RESTORATIVE ACTIVITIES OF *CURCUMA LONGA* ON SILDENAFIL INDUCED NEPHROTOXICITY AMONG MALE ALBINO RATS

(*Rattus norvegicus*)

BY

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DECLARATION

This research thesis is my original work and has not been presented for award of degree in any university.

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May almighty God bless you.

DEDICATION

This research thesis is dedicated to my lovely and beautiful daughter Tarshley Adelle, my parents Mr. Isaac Khisa my father and Mrs. Rose Nandako my mother, brothers Nesbit, Brian, Arnold and my sister Faith Khisa.

ABSTRACT

Nephrotoxicity is the leading cause of acute kidney injury worldwide with majority of population affected being of African population as it accounts for 18- 27% of acute kidney injuries managed in hospital set up. Its clinical manifestation includes acute and chronic injuries. Sildenafil is among the drugs postulated to affect the kidneys because it releases active radical scavenger chemicals that damage the renal glomerular, capsules and blood vessels. *Curcuma longa* commonly known as turmeric is used because of its high levels of antioxidants and anti-inflammatory nature. There is paucity of data on its pharmacokinetics, dynamics and restorative benefits, therefore the main objective of this study was to investigate restorative activities of *Curcuma longa* on sildenafil induced nephrotoxicity among male albino rats. Specific objectives being; to assess the gross histomorphological and stereological changes on kidneys on administration of sildenafil, to determine the different doses of *Curcuma longa* required to provide restorative effects in sildenafil induced nephrotoxicity, to evaluate the renal biochemical parameters of BUN and creatinine following administration *Curcuma longa* on sildenafil toxicity and lastly assess gross histomorphological and stereological changes on kidneys on administration of *Curcuma longa* in sildenafil nephrotoxicity. The study was a post-test only true experimental design, conducted at Maseno University in Kisumu County, Kenya. A total of 25 male albino rats of the species *Rattus norvegicus* were used and grouped into two groups as control or experimental. The experimental groups were subjected to sildenafil then *Curcuma longa* respectively at calculated doses. The ethical approval was sought from both the school of postgraduate research committee and animal research committee. Data was entered into excel spread sheet, analyzed using the statistical package for social sciences version 22.0. The kidney biochemical markers were compared before and after restoration using t-Test. One way ANOVA with post hoc Bonferroni was used and a P value ≤ 0.05 was considered significant. The mean body weight of rats in SIN group was statistically significant(P=0.0001) as compared to Control group and experimental groups. The mean weight of right kidney was slightly higher than left kidney in SIN group although their weight respectively was statistically significant (P=0.0001) as compared to control and experimental groups. The mean volume of right and left kidney in SIN group was statistically significant as compared to control and experimental group. The mean length and thickness in SIN group were statistically significant (P=0.0001) as compared to control and experimental groups. On histological changes in SIN group; the glomerulus was shrunk, bowman`s space dilated, proximal convoluted tubule dilated and epithelial cells appeared necrotic. In experimental groups, medium and high *Curcuma longa;* the glomerulus appeared normal, bowman`s space narrow, proximal convoluted tubule constricted and epithelial cells were normal and appeared cuboidal in shape. On stereological findings in SIN group; the absolute glomerulus volume was statistically significant (P=0.0001) as compared to control and experimental groups whereas the number weighted volume fraction of epithelial cell was statistically significant (P=0.0001) in SIN group as compared to control and experimental groups. The renal biochemical parameters in SIN group were statistically significant (P=0.0001) as compared to control group. In conclusion, Sildenafil citrate had toxic effects on kidney as demonstrated by the gross morphometrical, histological and stereological changes. Medium and high *Curcuma longa* dose were more effective as demystified by the gross morphological, histomorphological and stereological changes. There were no changes in biochemical parameters in SIN group as compared to experimental groups. Medium and high *Curcuma longa* doses were found to improve the gross morphometrical, histomorphological and stereological changes. Therefore, this research recommends that sildenafil has effects on kidney histoarchitecture when used at high dose or prolonged period of time and should be regulated. *Curcuma longa* has restorative effects on histoarchitectural changes caused by sildenafil and there is need to do further research to ascertain the pharmacokinetics and pharmacodynamics of the different components responsible for the changes.

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ABBREVIATION

AED : Animal Estimated Dose

AKI : Acute Kidney Injury

ANOVA : Analysis of variance

BUN : Blood Urea and Nitrogen

FGF : Fibroblast Growth Factor

H&E : Hematoxylin and Eosin

HED : Human Estimated Dose

HCLDG : High Curcuma Longa Dose Group

IGFBP : Insulin Growth Factor- binding protein

KIM : Kidney Injury Molecule

LCLDG : Low Curcuma Longa Dose Group

MCLDG : Medium Curcuma Longa Dose Group

NIAR-USA : National Institute of Animal Research of United States of America

NGAL : Neutrophil Gelatinase – Associated Lipocalin

SDG : Sildenafil Dose Group

SPSS : Statistical Package for Social Sciences.

TIMP : Tissue Inhibitor of Metalloproteinase

OPERATIONAL TERMS

Albino rats : Is a laboratory rat of species Rattus Norvegicus Domestica which is bred and kept for scientific research.

Histosterelogical : This is the study of microscopic anatomy of the cell and tissue in three-dimensional qualification of two dimensions cross section of exact quantitative information.

Histomorphology : This is the study of cell morphology, distribution and density using a microscope.

Morphometry : This is the process of measuring the external shape and dimensions of an object.

Terminal weight : This is weight before sacrificing is carried out.

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CHAPTER ONE

INTRODUCTION

1.1 Introduction and Background Information

Curcuma longa commonly known as turmeric, is a popular herbal plant mostly found in India and Southeast Asia, Kenya and some regions in the world (Omosa *et al*., 2017). It is used as traditional medicine in treatment of diseases including diabetes, dermatological diseases, arthritis, peptic ulcers, atherosclerosis, cardiovascular diseases, respiratory diseases and hepatobiliary diseases (Witkin & Li, 2013). 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% (M. Lestari & G. 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, a manufactured and prescribed drug occurs as suspension or tablet. It is readily available in market as Viagra or Vizarsin (Nichols *et al*., 2002). The chemical formula is $C_{22}H_{30}N_6O_4S$ and has bioavailability of 40% and normally excreted in feces or urine *(Nichols et al*., 2002). It is a safe drug for use with side effects ranging from mild to severe. 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).

Nephrotoxicity is kidney rapid deterioration in function due to the toxic effect of medicines and chemicals, environmental and industrial toxins as they affect the renal function differently (Lv & Zhang, 2019) . Drug induced nephrotoxicity is increasingly the major factor that

contributes to the development of kidney diseases as this type of nephrotoxicity has a wide range of damage on the nephron. Sildenafil has wide variant methods of nephrotoxicity as it 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). Albino rats are animals that are used in conducting invitro research. They were the first species to be domesticated and are normally of the species *Rattus Norvegicus*. The albino rats have the capacity to reflect the different changes when subjected to any drug because they have a close biological and functional association to human beings. The use of these rats is based on the following reasons; relatively low costs of maintaining the animals, plentiful and readily available. The animals are small in size, easy to handle and care during the experimental process. They have the ability to withstand a wide range of medicines used in studies (Bailey *et al*., 2014).

Curcuma longa has the physiological properties that can counteract, attenuate, ameliorate and protect the kidney from sildenafil induced nephrotoxicity. However, there is less data which shows the restorative effects of *Curcuma longa* on sildenafil induced nephrotoxicity. *Curcuma longa* is greatly able to protect the kidney from sildenafil induced nephrotoxicity in the following ways, it counteracts the tubular damages, apoptosis and oxidative stress released (He *et al*., 2015), counters the activities of heme oxygenase, endothelial nitric oxide synthase and gene expression of tumor necrosis factor alpha (Hassan *et al*., 2019) and it has Curcumin

that has anti-inflammatory and antioxidative effects thus able to counteract the reactive oxygen and nitric oxide radicals released (Ueki *et al*., 2013).

1.2 Statement of the problem

The incidence of acute kidney injury is increasing to epidemic proportions with its development leading to excessive morbidity and mortality. Moreover, it is associated with long hospital stay and high health care costs (Soni *et al*., 2009). It is a major contributing factor to poor patient outcome, affecting about 13.3 million people per year of which 85% of the affected are from developing countries (Mehta *et al*., 2015) . This high incidence is attributed to lifestyle changes, use of herbal medicinal, increase in environmental toxins because of industrialization and development of conditions like hypertension, cardiac diseases and diabetes. Drug induced nephrotoxicity accounts for 18-27% of all cases of acute kidney injury which is pegged on the clinical practice of use of drugs associated with renal dysfunction. Sildenafil as a cause of acute kidney injury cannot be overlooked based on the increase in reported cases of nephrotoxicity and increase in rate of its prescription. There is scarcity of data on restorative activities of *Curcuma longa* on histomorphological changes on kidneys due to sildenafil induced nephrotoxicity. Sildenafil damages the kidneys by releasing reactive oxygen and nitrogen radicals that damage the glomerulus, nephron and cortical tubules whereas *Curcuma longa* destroys the released free reactive radicals thus causing attenuation and counteracting benefits. Therefore, this research sought to determine the restorative activities of *Curcuma longa* and cross examination of histoarchitectural changes that occur on kidneys as an indicator of restoration.

1.3 Justification of the study

Sildenafil is the first line therapeutic agent for treatment of erectile dysfunction and pulmonary hypertension. It is commonly abused as an over-the-counter drug because of its sexual activity benefits. This study helps to show that *Curcuma longa* can help restore kidney histoarchitecture after it has been damaged by sildenafil based on its antioxidation activity therefore, it gives an insight on the use of *Curcuma longa* in restoration of sildenafil induced nephrotoxicity. This helps in reducing the incidence of renal diseases that is associated with sildenafil induced nephrotoxicity. *Curcuma longa* has reno protective, ameliorative, attenuation benefits however there is scarcity of data on its restorative abilities in AKI (Ghosh *et al.,* 2009; Tapia *et al*., 2013). The albino rats were used because of close biological, physiological and functional relations with human beings (Bailey *et al*., 2014). BUN and creatinine were used as key biochemical parameters because they have ability to diagnose AKI at earlier stages (Wasung *et al*., 2015).The study confirmed restorative effects of *Curcuma longa* on Sildenafil induced nephrotoxicity, this is beneficial to population using Sildenafil since *Curcuma longa* is readily available locally in the market.

1.4 Objectives of the study

1.4.1 Main objective

To determine the restorative activities of *Curcuma longa* on sildenafil induced nephrotoxicity among male albino rats**.**

1.4.2 Specific objectives

1. To assess gross morphological, histomorphological and histosterelogical changes that occur on kidneys on administration of Sildenafil among male albino rats.
2. To determine the different doses of *Curcuma longa* required to provide restorative effects in sildenafil induced nephrotoxicity among male albino rats.

3. To assess the gross morphological, histomorphological and histosterelogical changes on kidneys on administration of *Curcuma longa* in sildenafil induced nephrotoxicity among male albino rats.
4. To evaluate the renal biochemical parameters of BUN and Creatinine following administration of *Curcuma longa* on sildenafil induced nephrotoxicity among male albino rats.

1.5 Hypotheses of the study

1. There was significant gross morphometrical and histomorphological changes that occurred on kidneys on administration of Sildenafil among male albino rats.
2. There was significant difference in doses of *Curcuma longa* required to provide restorative effect on Sildenafil induced nephrotoxicity.
3. There was significant gross morphometrical, histomorphological and histosterelogical changes that occurred on kidneys after administration of Sildenafil then *Curcuma longa* among male albino rats.
4. There was significant change in levels of biochemical parameters of BUN and Creatinine in Sildenafil induced nephrotoxicity and on administration of *Curcuma longa.*

1.6 Study model assumption

The adult male albino rats were used because they have the ability to replicate similar effects of the drug as on human due to their close biological and functional associations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Curcuma longa and Sildenafil has been researched on worldwide. The literature on the physiological properties of *Curcuma longa* on how it attenuates, ameliorates and protects the kidney on sildenafil induced nephrotoxicity is readily available. This is because drug induced nephrotoxicity is the third leading cause of acute kidney injury.

2.2 Histomorphological changes that occur on kidneys on administration of Sildenafil

Histomorphology is the study of morphology of cells and tissues in terms of structure, size and shape with the help of a microscope (Pathak *et al*., 2017). When the kidney is subjected to different drugs it tends to change its histological structure. This is based on a study by (Adams *et al*., 2020) which shows that images from the cortical and medullary regions of kidneys had predominant changes when they were subjected to different drug dosage which implies that such a tissue has undergone tubular necrosis. A study by (Mohammed & Khudair, 2020) on histopathological changes on kidneys on administration of Sildenafil demystifies consistent kidney damage changes on cortical tubules and glomeruli. These changes of severity are variant including; cystic dilatation of cortical tubules, dilated bowman space, cellular degeneration, calcification and necrosis being the severest.

Sildenafil has cytotoxic effects which causes cytoplasmic vacuolations increased permeability of membrane, dilation of medullary tubules, proximal convoluted tubules and cortical tubules (Mshelbwala *et al*., 2019). It being cytotoxic, there will be increased intracellular accumulation of water in the cells due to cellular injury that tends to increase permeability of cell membrane (Aljeboori & Majhool, 2017). There is change in permeability of cell membrane due to cellular damage by oxidative chemicals (Ebrahimi *et al.*, 2009) and glomeruli calcification due to

increased deposition of calcium (Küçük *et al*., 2012). Sildenafil causes significant change in renal segmental structure, glomerular capillaries and medullary lumen by causing capillary dilatation and luminal obstruction (Cadirci *et al*., 2011).

Sildenafil causes acute renal failure in individuals by causing massive dilatation of cortical tubules, flattening of tubulo-epithelial cells, segmental degeneration and interstitial edema as evidenced in a 39-year-old man who took 100mg of sildenafil for two consecutive days (Liu *et al*., 2018). Sildenafil causes massive damages to kidney by inducing distortion and shrinking of glomerulus, widening of the bowman's space, cortico-tubular cytoplasmic vacuolation and basal laminal degeneration as evidenced by histological analysis (Mohamed Yousry *et al.*, 2016). Sildenafil causes voluminous changes on cell membrane that leads to cytoplasmic vacuolation which is as a result of lactate accumulation that increases osmotic pressure whereas the cell membrane damage is due to the oxidative effect of free reactive and nitrogen oxide radicals released (Mohamed Yousry *et al*., 2016).

Kidney is retroperitoneally organ located anatomically at the level of T12 to L3. Its renal parenchyma is composed of the outer cortex and inner medulla. The normal gross morphometric parameters of kidney include; length, width, shape, weight and volume. In human beings, the weight of kidneys differs as per gender with a male kidney weighing 125-175g and female 115- 155g, a length of 11-14cm, width of 6cm and thickness of 4 cm more so, each kidney is covered by a fibrous capsule that is dense, irregular connective tissue maintaining its shape. Sildenafil reduces growth of collagen fibers and smooth muscles (Gümüş *et al*., 2004).

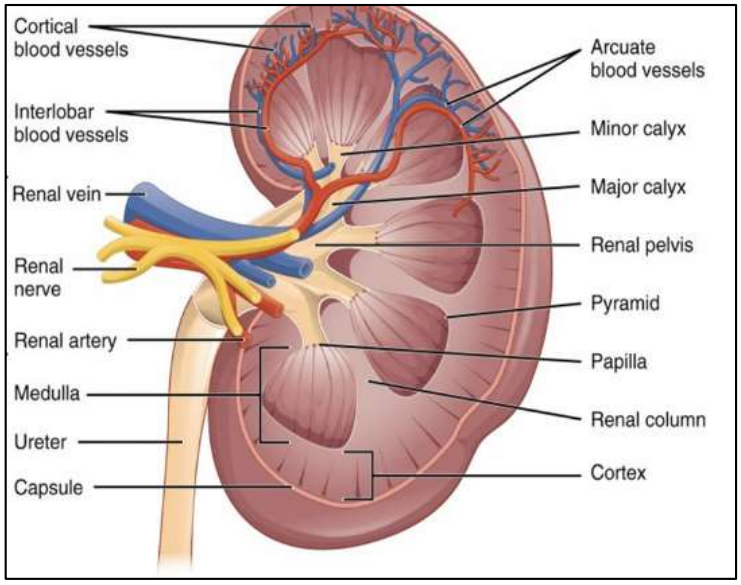

Figure 2. 1:*The figure above shows the gross morphometric anatomy of the kidney as adopted from:www.courses.lumenlearning.com.*

Medulla is the inner layer while cortex is the outer layer. The renal pyramids are usually arranged between the medullary layer and cortical layer. It has nephrons that are located within the cortex and medullar. The nephron is divided into four parts namely; loop of Henle, bowman's capsule, glomerulus, proximal convoluted tubules (Dreesen *et al.*, 2017). The cortical layer has renal corpuscles that contain proximal convoluted tubules, distal convoluted tubules and glomerular. The bowman's capsule has two layers (visceral and parietal) whereby they are the inner and outer layer respectively. Whereas the parietal layer and proximal convoluted tubule are lined with squamous epithelium, the visceral layer has glomerular capillaries (Vickers *et al.*, 2004).

The changes that occur on kidneys in humans and albino rats are similar. This is because of the close biological and functional associations of the two. The invitro experiments done on rodents demonstrates similar effects on human beings, therefore the pathophysiological changes that occur will consistently be same as seen in humans. The mediators that cause the disease will cause similar effects in different rodent models (Ali *et al.*, 2018). (Mohamed Yousry *et al.*, 2016) demystifies that sildenafil causes bowman's capsule dilation, cortical

tubule dilation, distortion and shrunken glomerulus. There is also cellular degeneration, calcification and necrosis (Mohammed & Khudair, 2020). This is based on the fact that sildenafil releases free reactive radicals that will interfere with the histoarchitecture of the kidneys. The histological changes that occur on kidneys, there severity is based on the dosage and duration of administration of sildenafil. Samples of kidneys analyzed showed prominent dilatation of proximal tubules and increase in interstitial spaces as evidenced in the works of (Suriyakumari *et al.*, 2016).

Table2. 1: *Comparison between various doses of sildenafil with histopathological effects adopted from Mohammed & Khudair 2020*

Group	**Sildenafil dose**	**Histopathological effects**
Group 1	1.5 mg/kg	Cystic dilation of cortical tubules, dilated bowman space and degenerated necrotic cortical tubules.
Group 2	2.5 mg/kg	Cystic dilation and glomerulus cellular degeneration and calcification.
Group 3	5 mg/kg	Degenerated necrotic tubules, massive infiltration of inflammatory cells and glomerular bowman space dilation.

(Kuno *et al.*, 2011) in their study on attenuation effects of Sildenafil found out that variant doses of Sildenafil cause varying effects of glomerulosclerosis, tubular dilatation, interstitial infiltration and fibrosis of the cortex in different ways whereby a semiquantitative analysis of sclerosis on glomerulus and tubulointerstitial fibrosis percentage was also done and results tabled as shown in table 2.

Table 2.2: *Comparison between various doses of sildenafil, % levels of glomerulosclerosis and % areas of tubulointerstitial fibrosis adapted from (Kuno et al., 2011)*

Groups of sildenafil.	**%Levels of glomerulosclerosis**	**% Area of tubular dilatation, interstitial infiltration and fibrosis of cortex.**
Group 1 concomitant	Sclerosis of up to 25%	Below 10% injured area
Group 2 0.1mg/kg	Sclerosis from 25-50%	Injured area from 11- 25%
Group 3 2.5mg/kg	Sclerosis from 50-75%	Injured area from 26-50%
Group 4 5mg/kg	Sclerosis of above 75%	Injured area from 50-76%

Sildenafil causes kidney necrosis this is based on a study that done to evaluate the effects of Sildenafil on adenine induced chronic kidney disease (Ali *et al*., 2018).

2.3 Effects of different doses of Curcuma longa on drug induced nephrotoxicity among albino rats

Most studies use a dose of 500mg- 2000mg of *Curcuma longa* in which curcumin concentration is very high. *Curcuma longa* is prepared in different formulation like powder, capsules and tablets with the latter two being the most common (Momenkiaei & Raofie, 2019). *Curcuma longa* is a multisystemic drug that is used in treatment of different conditions therefore the dose might differ based on the system being managed. In a assessing the renal protective effects of *Curcuma longa* among rats a dose of 75mg/kg/ day for 8 weeks was used by (Ghosh *et al.*, 2009). In further studies by (Soetikno *et al.*, 2011) rats were subjected to *Curcuma longa* dose of 75mg/kg for 8 weeks and it was established that *Curcuma longa* reduced systolic blood pressure and proteinuria which shows that *Curcuma longa* has ability to protect kidneys from any sort of damage.

(Tapia *et al.*, 2013) on-post treatment of renal disease with *Curcuma longa* at 120mg/kg for 30 days found out that *Curcuma longa* has reversal benefits on renal damage and oxidative stress. This study agrees with the works of (Correa *et al.*, 2013) where a dose of 120mg/kg for 67

days of *Curcuma longa* was found to be beneficial in management of cardiovascular diseases. In prevention of renal injury induced ischemia and reperfusion, *Curcuma longa* at 200mg/kg for 7 days is useful as it reduces levels of urea and cystatin C alongside increasing protein carbonyl content in kidney thereby, demonstrating its renal protective abilities. (Ueki *et al*., 2013) used 100mg/ kg of *Curcuma longa* in treatment of cisplatin induced renal injury in mice and he established that it prevents tubular necrosis and renal dysfunction thus improving kidney function. This study is in agreement with (Ali *et al.,* 2005) in which *Curcuma longa* at a dose of 200mg/kg for 10 days had ameliorative effects on gentamicin induced nephrotoxicity.

2.3.1 Curcuma longa components and the postulated restorative mechanism of the kidney cells

Curcuma longa is a traditional herbal plant that has curcumin as the active compound, ar-turmerone, beta sesquiphellandrene and curcumerol, desmethoxycurcumin, bismethoxycurcumin and cyclic curcumin (M. L. Lestari & G. Indrayanto, 2014). Curcumin has wide range of uses as it is used in treatment of respiratory diseases, cardiovascular disease, atherosclerosis and psoriasis. Its use in management of renal diseases cannot be overlooked because of the invitro experiments done that have demonstrated its attenuation, amelioration and nephroprotective effects. *Curcuma longa* has a chemical formula of $C_{21}H_{20}O_6$. It facilitates hydrogen reactions which is critical as it leads to hydrolysis, degradation and enzymatic reactions. *Curcuma longa* has excellent scavenging power for molecular and radical oxidants. These radicals will play a role in hydrogen destruction and transfer of electrons. Peroxyl radicals react with curcumin to produce Curcumin phenoxy radicals that are less reactive thus protection against ROS- induced oxidative stress (Borsari *et al.*, 2002).

Metabolism of Curcumin in rats produces is simply discusses in two different pathways as either reduction or conjugation (Wahlström & Blennow, 1978). Presence of Curcumin sulfate and tetrahydro Curcumin facilitates enzymatic reactions to take place rather than hydrolytic

degradation that is slow (Garcea *et al.*, 2004). Nucleophilic addition reaction of Curcumin: diketo moiety of curcumin participates in nucleophilic reaction termed Micheal addition. Curcumin forms the Curcumin -glutathione conjugates that leads to reductions in levels of intracellular glutathione leading antioxidant defense (Pandya *et al.*, 2000).

2.3.2 Mode of action of sildenafil and therapeutic benefits

The oral tablet is the one that is easily abused because it is more common and readily available in market with a brand name of Viagra (Nichols *et al.*, 2002). It is a phosphodiesterase type 5 inhibitor (Mulhall *et al.*, 2020), that works by prolonging signaling effect of nitric oxide thereby raising the cyclic guanosine monophosphate levels which causes the blood pulling effect in the pennis leading to an erection(Mohammed & Khudair, 2020). It has a bioavailability of 40% with rapid first pass metabolism, half-life of 4 hours (Grossman *et al.*, 2004) and its detoxification occurs in the liver by the two isoenzymes of the cytochrome P 450 enzymes pathway (Lue, 2000) and normally eliminated and excreted in feces and urine when an oral dose is taken in 80% and 13 % respectively (Nichols *et al.*, 2002). It has a maximum plasma concentration of 30 to 120 minutes with a 96% bound to plasma protein (Boolell *et al.*, 1996).

It is used in management of benign prostatic hyperplasia. In females this drug is used to improve uterine perfusion and venous return in those with ovarian torsion and it is known to inhibit cardiac hypertrophy and prevent heart failure (Ala *et al.*, 2021). In this case therefore, erectile dysfunction is a condition in which the penis of a man does not harden and expand or does not sustain an erection when sexually excited. It's tendency to be abused is high because of ability to increase sexual activity therefore, its adverse cardiovascular effects should be advised and the drug taken at specified time and dosage (Wang *et al.*, 1999). There are over 240 deaths associated with sildenafil out of which 128 are verified and as of 2008 (Giuliano *et al.*, 2010). It has a multi systemic benefits whereby the drug will serve a specific function for every system Cases of nephrotoxicity being reported after use of sildenafil continues to increase

with a recent incidence of a 67-year-old man who was on management of erectile dysfunction reportedly developed acute kidney injury upon taking 400mg of sildenafil within four hour (Liu *et al.,* 2018).

2.4 Renal biochemical markers level in sildenafil induced nephrotoxicity and on administration of Curcuma longa

Kidney biomarkers are the chemicals used to determine any decrease in kidney function and estimate both severity and nature of kidney injury. These biomarkers include lipids, proteins, genes, metabolites and cells present on urinalysis. Drug induced nephrotoxicity remains the leading cause of AKI. Majority of the population affected being from industrialized countries and this is attributed to the excess environmental pollution witnessed, life style changes that has led to the development of HTN, DM and CDS. Therefore, there is a high need of increase sensitivity and specificity of different tools, need to combine different biomarkers so as to increase the diagnostic accuracy (Wasung *et al*., 2015) used to detect drug induced nephrotoxicity as a cause of AKI at early stages. The emerging new biochemical markers include: KIM-1, KIM-2, TIMP-2, IGFBP7, NGAL, Cystatin C and FGF-23 (Wasung *et al.*, 2015). Serum creatine remains the major kidney biomarker however, it is associated with challenges like patients with low muscle bulky and fluid overload it may be hard for you to use Serum creatinine as biomarker therefore, there is need to combine with Cystatin C so as to improve its accuracy.

Sildenafil has been associated with nephrotoxicity because of increase in rate of reports of patients developing AKI upon its intake. It has cytotoxic effects as it's known to damage cell membrane. It also increases levels of lactate, calcium thus causing calcification and necrosis of tubules. In human beings' sildenafil causes increased blood urea nitrogen and serum creatinine levels. This is based on the management of 67-year-old man who developed AKI upon taking 400mg of sildenafil within four hours who on laboratory analysis on admission

was serum creatinine of 1.94 mg/dl and BUN of 16mg/dl. On days 4 and 6 the patient had a serum creatine level of 5.07mg/dl and BUN of 71 mg/dl respectively (Liu *et al.,* 2018).

Curcuma longa contains high concentration of Curcumin that have antioxidant benefits. In a study to evaluate effects of *Curcuma longa* on renal function test, rats were co-treatment with 12.5mg and 25mg/Kg respectively of curcumin. It was observed that there was a significant reduction in serum creatinine and urea levels with prevention on serum electrolyte derangements like potassium, sodium, chloride and hydrogen carbon (Akinyemi & Adeniyi, 2018).

Curcuma longa causes a significant decrease of BUN, serum creatinine and creatinine kinase levels that indicates curcuma has Reno protective benefits based on study to evaluate the effect of curcumin on glycerol induced AKI (Wu *et al.,* 2017). This study is in agreement with (Venkatesan *et al.,* 2000) in which rats were subjected to doxorubicin and treated with curcumin, on evaluation of biochemical markers there was a significant reduction in urine levels of N- Acetyl -B- glycosaminidase (NAG) which is normally a marker of tubular damage. In a study on reno protective effects of curcumin on cyclosporin A induced nephrotoxicity, the researcher establishes that curcumin attenuates renal morphology, antioxidant enzymes and renal function that was altered by treatment of cyclosporin A was normalized (Tirkey *et al.,* 2005).

2.5 Histomorphological changes that occur on kidneys on administration of Curcuma longa

Curcuma longa exhibits both ameliorative, attenuative and nephroprotective benefits on kidneys. This is based on antioxidative and anti-inflammatory benefits of *Curcuma longa*. *Curcuma longa* is known to produce an array of enzymes that tend to interfere with the nephrotoxic pathways of different drugs and metals. This is highly demonstrated in three

studies as follows; (Ahmad *et al.*, 2020) in a study to determine the benefits of *Curcuma longa* on streptozocin induced diabetes, on kidney histological analysis he found that the renal corpuscles were intact and there was minimal renal tubular degeneration. A study by (Ilyas *et al.*, 2019) demonstrated that *Curcuma longa* reduced levels of damage of proximal convoluted tubule alongside decreasing kidney necrosis, which indicates that *Curcuma longa* has the ability to maintain and improve the structure of cells of kidneys in rats. Furthermore, *Curcuma longa* has ability to significantly decrease the renal tissue damage by protecting glomeruli, Interstitium hyperemia and degeneration of renal tubular epithelial cells as evident in a study by (Liu *et al.*, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

This research was conducted in Maseno University, a public University located in Maseno Township along Busia Road,25 km from Kisumu City in Kisumu County Kenya. The institution offers both scientific and arts courses with well-established scientific laboratory and research committee. All experiments that pertain to handling, weighing, administration of Sildenafil, *Curcuma longa* and harvesting of kidney tissues was done at the department of Zoology because of the established animal house where the rats were kept. Tissue preparation, processing for histological study and analysis were done at the histology laboratory in human Anatomy department due to availability of expert and equipment. The renal biochemical markers were done at the Nairobi University department of Vetenary medicine (clinical chemistry) because of their well-established laboratory and equipment. The photomicrographs were taken in school of medicine at Jomo Kenyatta University of Science and Technology as they have an Olympus microscope.

3.2 Study design

This was a post-test only true experimental study design in which an intervention was implemented to compare results against control group.

3.3 Study subject

This experiment was carried on albino rats of the species of *Rattus norvegicus* that were purely bred from department of zoology whereby the animal house can hold a maximum of 250 rats in a given research period. The albino rats were bred in cages which can hold a maximum of 6 rats per cage as the 250 rats are classified in different research group. By appearance, the eyes are red, white in color and have close resemblance with the ‘’Japanese hooded rats’’ hence may

have a common ancestral origin as they are genetically identical (Pritchet and coming 2016). Have an average lifespan of three years and rapidly develop at infant period. These animals mature sexually at around 4-5 weeks in females while 45-48 days among males. Adult female rat weigh 350-450 grams while male can weigh between 450 and 650 grams respectively. Based on length, male rats are longer as compared to female rats at about 9 to 11 inches.

Figure 3. 1*: An illustration of a male albino rat (rattus norvegiccus)*

3.4 Sample size determination

Modified resource equation method was used in calculation of sample size as there is no research done on determination of standard deviation (Arifin & Zahiruddin, 2017) .

$$\boldsymbol{n = DF/k + 1}$$

n= represent animal number per group

k= group number

DF= degree of freedom

DF range from 10 to 20 is used as a way of obtaining the maximum and minimum number of animals per each group.

Therefore, sample size per group was calculated as

$\frac{20}{5} is\ 4 + 1 = 5$

Total number of subjects

$\boldsymbol{N = k\ X\ n}$.

$N = 5x5 = \mathbf{25}$

3.5 Sampling method

Here, 25 rats were randomly picked from the 250 albino rats and assigned to two major group as either experimental or control using the random simple sampling method.

3.6 Selection criteria

3.6.1 Inclusion criteria

- Health albino rats since their introduction into the polycarbonate cage.
- All the animals (albino rats) that would have attained the desired weight by the time of conducting the experimental study.

3.6.2 Exclusion criteria

- All the sick and weak animals.

3.7 Grouping of animals

In assigning the 25 animals, simple random sampling was used to allocate them into respective groups. The 20 albino rats in experimental group were further grouped into four groups each based on dose used, hence,5 rats for a low *Curcuma longa* dose (LCLDG3), 5 rats for medium

Curcuma longa dose (MCLDG4), 5 rats for high *Curcuma longa* dose (HCLDG5) and lastly 5 rats for group 2 that was only subjected to Sildenafil (SDG2).

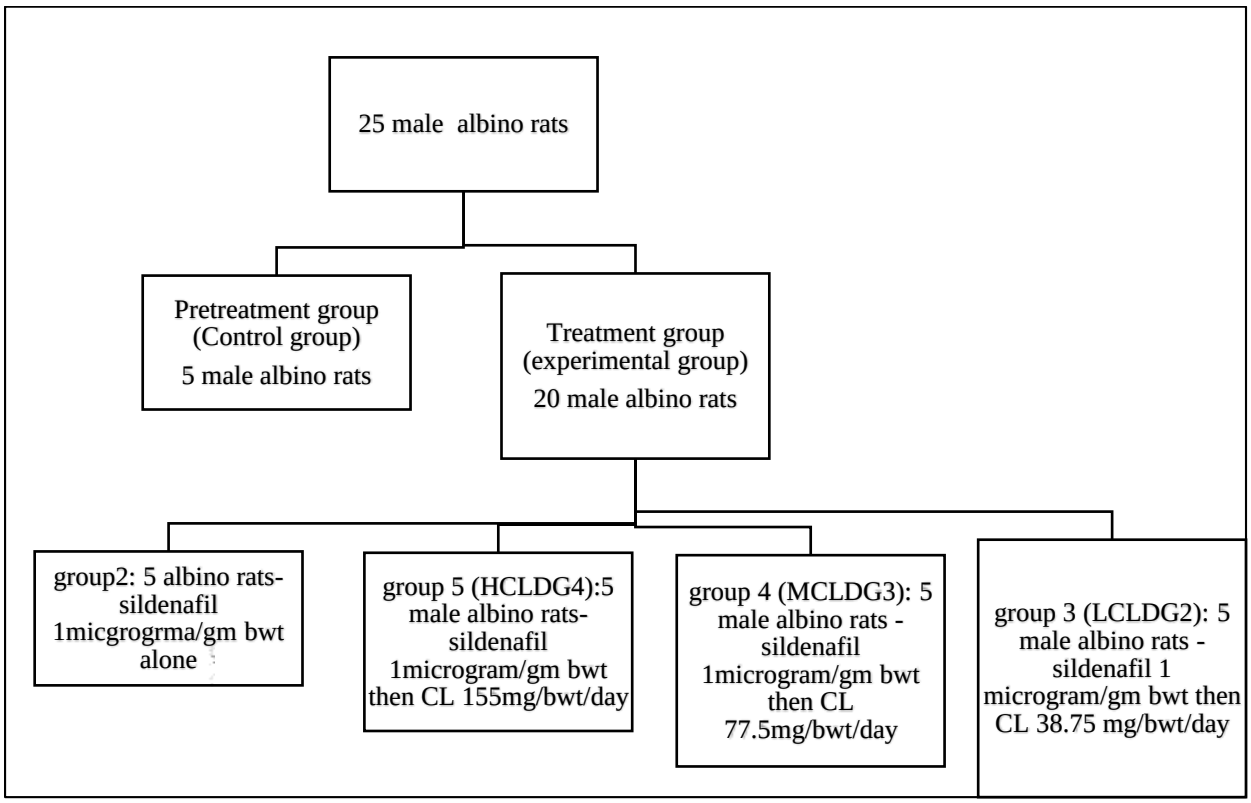

Figure 3. 2*: Grouping of rats*

3.8 Feeding of the rats

To enhance nutrition of rats, they were fed on rodent pellets that was obtained from Kisumu Poultry Centre from Kisumu City and water *ad libitum.* This was done every morning inside polycarbonate cages that are spacious and all rats stayed in these cages to allow acclimatization take place. The feeding was done according to two broad groups of control and experimental studies until they attained the required weight.

Figure 3. 3: *Feeding of rats*

3.9 Handling of animals

In order to obtain daily weights, cleaning of cages, head count to ascertain numbers per group, feeding and administration of drug was done between 0800 hours and 1000 hours by investigator alone. All the procedures of feeding were undertaken based on National Institute of Animal Research (NIAR-USA) and (Gomez *et al*, 2010) that clearly stipulates protocols and guideline on how to conduct them. Occupational safety precautions were adhered to fully when handling rats so as to avoid any injuries and spread of infections from rats to the investigator and vice versa. These was achieved through putting on laboratory coats at all times, glass eye protectors, gloves and closed shoes. Washing of hands was done before and after entry into the animal house and any procedure.

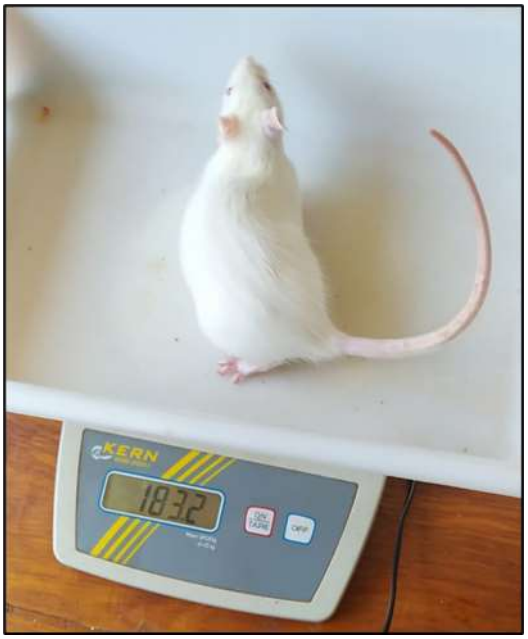

Figure 3. 4: *Weight of albino rat taken using Scout pro model EMB 1200-1*

3.10 Renal biochemical parameters assay before and after administration of Sildenafil

The renal biochemical marker assays were determined before administration of sildenafil in order to ascertain that albino rats have a normal kidney function. Sildenafil was administered then biochemical marker assays were done before administration of *Curcuma longa*. Here BUN and Creatinine were used to assist in early diagnosis of acute kidney injury.

Figure 3. 5: *A collected sample of blood for renal parameters and a centrifuging machine*

3.10.1 Sample collection of blood for renal biochemical parameters analysis

Collection of blood samples from rodents is important for in-vitro and in-vivo studies. The sites varies but they may include; jugular veins, maxillary vein, saphenous vein and the heart. In order to collect large amounts of blood, Cardiac puncture may be used as an average of 10ml blood can be collected from a rat weighing 150g (Beeton *et al*., 2007).

The procedure includes;

Prepared a 5 ml syringe with a gauge 23 needle, deeply anesthetized the rat, checked for anesthesia by lack of spontaneous movements or response to stimuli, placed the rat on its back facing away from you, placed the left index finger at the level of lowest ribs, without applying pressure. (Heart was located 1 cm above this point and slightly to the right), held the syringe at 45-degree angle inserted the needle between two ribs and watched for a drop of blood to come into the needle, a total of 3- 5 ml of blood was collected and immediately euthanized the rat.

Figure 3. 6*: Cardiac puncture conducted after the rat had been anaesthetized.*

3.11 Acquisition and determination of drugs

The *Curcuma longa* tablets (***Batch No:8#561748***) were acquired from Bio health MY DAWA online pharmacy in Nairobi City under a trade name of Turmeric Rhizome. Sildenafil tablets (***Batch No:5810494***) were obtained from OPANGMED pharmacy under a trade name of Viagra. All the products were approved by the pharmacy and poisons board (PPB) and had respective serial numbers.

3.11.1 Determination of sildenafil doses for the experiment

Sildenafil dose of 1microgram/gm bwt was used (Suriyakumari *et al.,* 2016).

3.11.2 Determination of doses of Curcuma longa and sildenafil

The calculation was based on the explanation of (Nair & Jacob, 2016) in a sample guide that provides guideline on how to convert an animal dose from human. This pharmacological guideline explains that the dose is related to body weight equally. Average human weight of 60 kilograms and body surface area of 1.62m^2. Km factor was used in determining the Human Estimated Dose although it varies in various animal species.

HED mg/kg= animal dose mg/kg/ animal K/human Keq.

The Km factor of each species is constant; therefore, Km ratio is used to simplify the calculations.

Whereby equation 2 will be HED mg/kg=animal dose mg/kg K ratio Eq.

Km ratio values are obtained by dividing human Km factor by animal Km factor.

3.12 Calculation of doses

The normal human single standard dose of *Curcuma longa* extracts is 1500mg*(Fança-Berthon et al.,* 2021). The maximum *Curcuma longa* human dose is 1500mg/day, medium dose of 750 mg/day and minimum dose of 375mg/day.

3.12.1` Calculation of high curcuma longa dose

The maximum human *Curcuma longa* dose per day is 1500mg and the average weight of a man is 60kg.

1500mg= 60kilogram

? =1kg

1x1500/60=25mg/day

AED=HED x Km factor

AED=25x 6.2= **155mg/kg/day**

3.12.2 Determination of medium Curcuma longa dose

Medium *Curcuma longa* dose is 750mg/day.

750mg=60kg

? =1kg

1x750/60=12.5mg/day

AED=HED x Km factor

AED= 12.5x6.2=**77.5mg/Kg/day**

3.12.3 Determination of low *Curcuma longa* dose

Lowest *Curcuma longa* dose is 375mg/day.

375mg=60kg

? =1kg

1x375/60=6.25mg/day

AED=HED x Km factor

AED=6.25x6.2=**38.75mg/Kg/day**

155, 77.5 and 38.75mg *Curcuma longa* tablets and deionized water were used to make the recon strictions. Then the animals received as follows;

1. **155mg/Kg/day** for the High *Curcuma longa* dose group (HCLG).
2. **77.5mg/Kg/day** for the medium *Curcuma longa* dose group (MCLG).
3. **38.75mg/Kg/day** for the Low *Curcuma longa* dose group (LCLG).

3.13 Administration of *Curcuma longa* and sildenafil doses

Administration of Sildenafil and *Curcuma longa* was done daily between 0900 hours and 1000 hours.

3.13.1 Materials needed

Curcuma longa, sildenafil, 20 albino male rats, gavages tube gauge 18, 5 ml syringe, table cloth, deionized water and beaker for dilution. A maximum of 3 ml of dissolved drug was administered daily.

3.13.2 Method of administering Curcuma longa and Sildenafil

Curcuma longa and Sildenafil were administered through gastric gavage.

3.13.3The procedure followed in administering various doses of Curcuma longa and Sildenafil

Identified neck region of the animal and held carefully using the left hand, it was then wrapped with a table cloth so as to avoid contaminating the investigator, the animal then rested against the body with mouth facing the investigator, gently introduced the gavage tube into mouth turning it slowly and gently to facilitate the tube pass through the three respective esophageal constrictions, Sildenafil and *Curcuma longa* dose were dropped into the stomach and gavage tube was carefully and slowly pulled off.

3.14 Humane killing of the albino rats and harvesting kidneys

3.14.1 Materials

25 male albino rats, concentrated carbon dioxide, cotton wool, specimen collection bottles, fixatives, formalin solution, scalpel, scalpel holder, dissector jar, electronic weighing machine, mounting board, mounting pins, hypodermic needle gauge 20, magnifying glass, surgical gloves, pair of scissors, pair of toothed forceps.

3.14.2 The procedure for anaesthetizing albino rats

For humane killing of animals, concentrated carbon dioxide was used, the gas was allowed to flow into jar that had a tight fit so as to prevent it from leaking out into the air, Albino rats were put into the jar for 3-5 minutes to euthanize and after euthanasia the rats were mounted on wooden board with pins and the back rested on the board while abdomen was facing upwards. A xiphoid process to pubic symphysis cut was made using a pair of scissors and forceps, Kidneys were identified, the whole kidneys was excised and tissues were put in beakers with formalin solution for 24 hours.

3.15 Assessment of the gross morphometrics of the kidney

The gross morphometrical parameters of kidney included thickness, width, length, weight and volume. 5% normal saline was used in cleaning the resected kidney. Thereafter, the thickness, width and length were determined using the digital vernier caliper and ruler. In determination of percentage kidney body ration the formula below was adopted.

Percentage kidney by ratio= kidney weight / animal weight in grams' x 100%

Figure 3. 7*: Shows weighing of kidney (taken using Scout pro model EMB 1200-1).*

3.15.1 Evaluation of the total kidney volume using Archimedes principle

Estimation of kidney volume was done immediately after dissection and removal of kidney by use of the immersion method basing on the principle of Archimedes as explained by (Cavazzini, 2019) . Here a well calibrated beaker was used and filled with 5% normal saline. The kidney was then dropped into this beaker and the amount of normal saline displaced represented the actual volume of the kidney dropped in. The volume acquired was compared with volume generated with other methods and the mean standard deviation of the measurement was determined.

Figure 3. 8*: Showing gross morphometrics, electric digital Vanier caliper of serial number 382371.*

3.16 Processing of kidney tissues for light microscopy

Fixation of tissues was done using the formaldehyde solution for 24 hours, Dehydration of samples was done using ethanol that had been prepared in an ascending concentration to a maximum of 100% with each concentration lasting one hour, Clearance of kidney sample were done using xylene, The sample tissues are then infiltrated with wax for twelve hours at 56^0c and Orientation of tissues was then done by making longitudinal cuts from the apex to base. At this point, the samples were embedded in paraffin wax when they had been put on the wooden blocks, to properly expose the whole kidney tissue, all the excess wax was chopped off from the blocks, rotary microtome was used to cut prepared sections into 5µm thick longitudinal sections and to properly spread the tissue, the cut sections were allowed to float in water at thirty-seven degrees. The sections were then put on top of the glass slides and a thin film was applied over it by a micro- dropper, using an oven, the slides were dried at thirty-seven degrees for 24 hours, slides were then taken through the procedural staining with Hematoxylin and eosin (H&E) and selection of slides for viewing under the light microscope was done through simple random sampling method.

3.17 Materials for staining

Kidneys, specimen bottles, Zenker solution, distilled water, acetic acid, DPX mount ant, glass slides, cover clips, glass staining square jar, hematoxylin, eosin, wax paraffin, knives, rotary microtome, slide holders, heater, water bath container, formaldehyde 40% concentration, xylene, isopropyl alcohol, glass ware for preparing dilutions, wood blocks, beakers, dropper and toluidine solution.

3.18 Materials and procedure used in photography

3.18.1Materials

1. digital 32 Megapixel camera.
2. 16 GB flash disc

3.18.2 Steps in taking photomicrograph using a digital 32-megapixel camera

Mounting of prepared slides on a microscope stage, adjust the focus of the microscope to ensure that the image to be taken in a clear focus, appropriately adjust the field magnification and only best viewed images were taken based on the proper focus as generated by the microscope. Transfer the photographs taken to the computer using the flash disc and by use of adobe fireworks all the images taken are labelled and uploaded in the program me component of the computer.

3.19 Processing kidney tissue for histo-stereological Analysis

3.19.1 Preparation of tissues for stereology

Once kidney tissue had been obtained, it was prepared diligently for stereological analysis. To allow proper fixation of these tissues they are placed in a solution of formalin for twenty-four hours normally at a room temperature of twenty-three degrees Celsius. The slides were then dehydrated in ethanol at an ascending concentration of ethanol of 50% to 100% lasting one hour at each concentration. They were then cleared with xylene for twelve hours. Paraffin infiltration of kidney tissues were done for 12 hours, thereafter be embedded in paraffin wax. A microtome sledge was used to facilitate in cutting of the tissues at thickness of 5µm.

3.19.2 Staining of kidney slides

The procedure of staining kidney sections was done using the hematoxylin and eosin solutions as described by Ghosh *et al.,* 2014.

3.19.3 Procedure for staining with hematoxylin and eosin

Place the glass slides with paraffin sections on staining racks, clean the paraffin from samples using xylene by dipping the slides in it three times at two minutes on every dip, the kidney samples were Hydrated using the following steps, slides were transferred through three steps of 100% ethanol whereby at each step lasted at least two minutes, they were then transferred to 95% ethanol for at least 2 minutes and after the two minutes of staying in 95% ethanol, the slides were then transferred to 70% ethanol for 2 minutes. The slides were washed in running water tap at room temperature for at least two minutes.

Thereafter, hematoxylin solution was used by dipping the kidney sample slides for three minutes, they were then washed with running water for 5 minutes at a room temperature. Then sample slides were stained using eosin Y solution for two minutes, the eosin Y-stained slides were then taken through the dehydration process as follows; The slides were put in a 70% concentrated ethanol for a total of twenty minutes, they were transferred to 95% ethanol beaker for two minutes, It was then taken through two steps of 100% ethanol with each step lasting for two minutes, the samples are then washed three times with xylene for 2 minutes per every single turn, Place a drop of Per mount over a tissue placed on each slide and a coverslip was added to ensure that the tissue was firmly held on the glass slide and they are then dried and therefore deemed ready to be viewed using a light microscope.

3.20 Estimation and determination of kidney volumes and histological changes

3.20.1 Estimation of kidney volume by Cavalieri method

Immediately when done with the procedure of processing the slides, the processed slides were selected from each kidney by the use of a simple random sampling method. The identified slides were thereafter be kept in an oven at 37^0 C for approximately twelve hours. These slides were then be viewed by use of a light microscope. Thereafter, the selection of microscopic

fields was done randomly in each kidney section. Then, the stage of a microscope was moved in X and Y directions every time, until the whole section has been studied. The taking photos was done using LABOMED ivu 3100 imaging camera softener using the pixel pros. The results obtained were entered on excel spread sheet.

3.20.2 Procedure used to determine the kidney total volume when using the Cavalieri principle

1. Use of a quantitative stepanizer in analysis of kidney tissue images.
2. Points that are over the kidney were identified and be marked.
3. Every point was counted
4. The volume of the total kidney was determined using this formula

Total volume= area of point by thickness x interval of the section

The steps and formula used to determine the volume densities of kidneys is described below.

Prepare the kidney slides to be used in Cavalieri, Select the spacing to be used for the point probe, toss randomly the point probe over each section, Points that hit the region of interest were considered and counted by use of the Stepanizer, Processing of all sections were done at this point. A tally of counts based on each section were kept, Estimation of the shape factor were done, the volume and coefficient Error were estimated and final volume of kidney slides were determined by using the following formula:

Volume = t x a/p ÷m ΣP

t- represents the thickness of kidney section.

a/p- shows the area of each point on grid counting point.

ΣP-is the total number of all the points that hit the area of interest.

m- it is the magnification.

3.21 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.

$V=\sum P \times (a/p) \times t$

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

$Ps=P/A \times 100\%$

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=$\sum P/A/n \times 100$

Where n= number per samples.

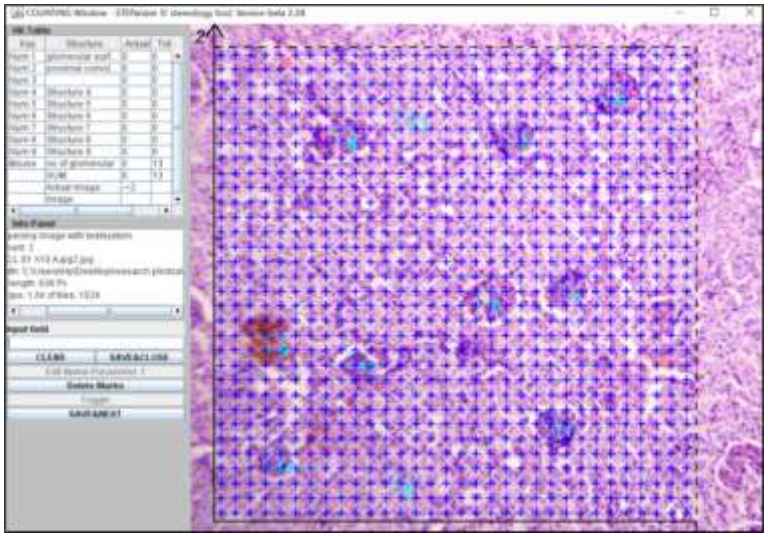

Figure 3. 9: *Illustration of stereological analysis; Cavalieri principle by stereology tool, version beta 2.28*

3.21 Correction for tissue shrinkage during stereological analysis

The stereological size is usually affected by shrinking which normally occurs when conducting a histological experiment and fixation procedures. The volume and density are some of the parameters that are highly affected. When determining the volume shrinkage, it was prudent to consider volume of a fresh kidney as determined by immersion method and later consider the volume as obtained by the Cavalieri principle. In determining this volume, the formula below was applied.

Volume shrinkage=1- (volume as per principle of Cavalieri / volume obtained using displacement method)

Volume shrinkage = 1 – [volume after / volume before)

3.22 Data analysis

Data was entered into excel sheet, analyzed through Statistical package for social sciences (SPSS)Version 25. Analysis was done as per objectives. Data concerning gross morphometrical analysis was calculated in mean, variance, median and percentage and be presented in bar graphs or histographs. The biomarkers were compared before and after

restoration of the kidneys using t-Test. One -way ANOVA with post hoc Bonferroni was further used to compare the data obtained from experimental and control groups where P- value ≤ 0.05 was considered significant.

3.23 Ethical Approval

Once approved by the school of medicine the document was forwarded to the school of graduate studies for necessary consideration. It was then be forwarded to East Africa University of Baraton committee of animal ethics for further approval and National Thereafter, the researcher underwent training on how to handle, feed and administer drugs as per the animal research and ethics committee. All procedure that pertains to this study were carried out based on guidelines and protocols for use and care of animals in biomedical research 2016. The animals were sacrificed in a humane way based on prescribed protocol as per studies of (Leary *et al.,* 2013).

CHAPTER FOUR

RESULTS

4.1 Introduction

A total of 25 adult male albino rats of between the weights of 150g and 250g were randomly selected for the study. Each rat was allocated to the 5 groups as either pretreatment group or treatment group. Every group received a total of 5 rats (20%). They 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 damage caused. The following results were observed from the study.

4.2 Body weight, Behavioral changes and mortality report

The average body weight of 25 rats used at commencement of the study was 183.75g with a mean standard error of ±3.14g. Rats in all the 5 selected groups did not show any behavior changes during routine inspection. There were no observable signs of nephrotoxicity during and after administration of drugs. No mortality was recorded during the entire process of the experiment.

4.3 Gross histomorphological and stereological changes on kidneys on administration of sildenafil

4.3.1 Gross morphometric findings of the kidney

4.3.1.1 Anatomical findings and macroscopic demonstrations of a kidney

On external morphological observation, the right kidney was cranially placed than left and related to the liver while left was related to stomach, pancreas and spleen which is a normal anatomy. The internal parts were cortex, medullar and renal pelvis respectively. It had the concave and convex margins. Figure 4.1 plates 1,2,3 and 4.

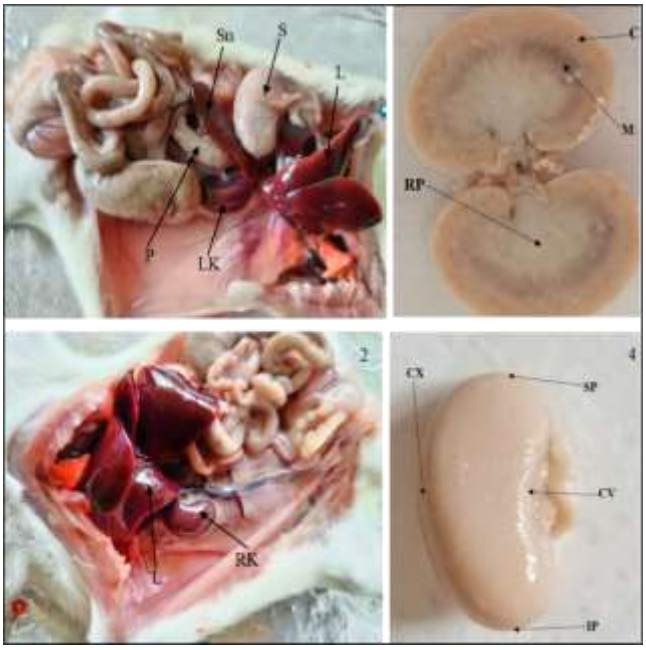

Figure 4.1: *plate 1 and 2, showing relations of left and right kidney respectively,3 shows internal parts of kidney while 4 represents the gross morphometrical cardinal points.*

KEY: *Sn= spleen, S=stomach, L=liver, P=pancreas, LK=left kidney, C=cortex, M=medullar. RP=renal pelvis, RK=right kidney, CX=convex, CV=con cave, 1P=inferior pole and SP=superior pole.*

4.3.1.2 Mean terminal body weight (final body weight before sacrificial), mean weight of right and left kidney and mean volume right and left kidney among control groups

The mean terminal body weight of rats in positive control (Sildenafil 1µgm/gm bwt/day) group was statistically significantly different (*P*=0.0001) when compared to negative control group (feeds + water). The mean weight of right and left kidney in positive control (Sildenafil 1µgm/gm bwt/day) group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) when compared to the negative control (feeds + water a) group respectively. The mean volume of the right and left kidney in positive control (Sildenafil 1µgm/gm bwt/day) group was statistically significantly different (*P*=0.0001, *P*=0.0001) when compared to the negative control (feeds + water) respectively (Table 4.1).

Table 4. 1: The mean terminal body weight, mean kidney weight and volume among control groups.

Mean body weight, kidney weight and volume in gms and mls					
Groups	**Mean terminal body weight**	**Mean weight right kidney**	**Mean weight left kidney**	**Mean volume right kidney**	**Mean volume left kidney**
Control (feeds + water ad libitum)	284.78±.32	1.16±.08	0.92±.02	1.93±.03	1.54 ±.05
SIN 1µg/gmbwt/day	254.74±.81	0.94±.02	0.82±.04	1.58 ±.04	1.14 ±.02
P value	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

KEY: *All values are expressed as the mean± 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.*

4.3.1.3 The mean length, width and thickness between negative and positive control groups

The mean length of right and left kidney was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) in positive control (sildenafil 1µg/gmbwt/day) group as compared to negative control group (feeds+ water). The mean thickness of right and left kidney was statistically significantly different (R; *P*=0.0001 L; *P*=0.0001) in positive control (sildenafil 1µg/gmbwt/day) group as compared to negative control group (feeds+ water) respectively (Table 4.2).

Table 4. 2: The mean length, width and thickness between negative and positive controls

Kidney gross morphometries in Mm.						
Groups	**Mean right kidney length**	**Mean left kidney length**	**Mean right kidney width**	**Mean left kidney width**	**Mean right kidney thickness**	**Mean left kidney thickness**
Control (feeds + water ad libitum)	18.57±.47	15.43±.55	11.59±.79	9.83±.57	5.66±.28	4.01±.02
SIN 1µg/gmbwt/day sildenafil	14.16±.36	11.97±.58	10.27±.16	9.66±.22	3.03±.07	2.91±.02
P value	**0.0001**	**0.0001**	0.903	1.000	**0.0001**	**0.0001**

KEY: *All values are expressed as the mean± 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*

4.3.2 Histological findings in negative control and positive control groups

In negative control group (feeds + water ad libitum), the glomerulus was normal and surrounded by a narrow bowman`s space. The epithelial cells were normal along the proximal tubule with well-defined margins and lumen had normal brush borders. In positive control group (Sildenafil 1µg/gmbwt/day), the glomerulus was distorted with a dilated bowman`s space, dilated cortical tubule with deranged borders, vacuolations, necrotic epithelial cells and lumen had distorted brush borders (Figure 4.2).

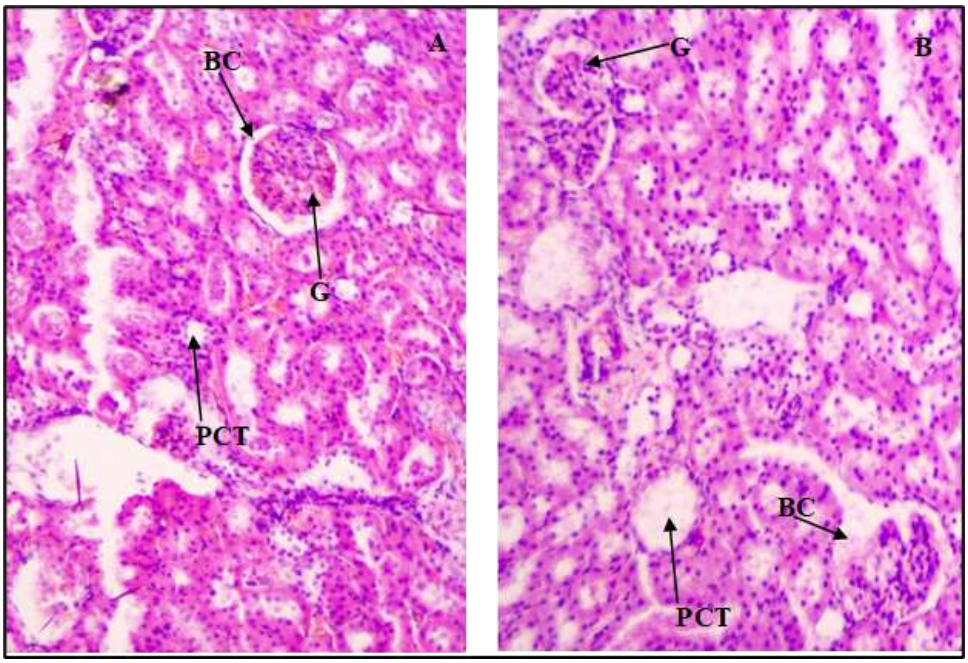

Figure 4.2: *Photomicrograph A, control showing normal glomerulus, normal epithelial cells, bowman`s capsule and proximal convoluted tubule. B, in Sildenafil induced nephrotoxicity showing distorted glomerulus, dilated bowman`s capsule and cortical tubule with necrosis.*

KEY: *G=glomerulus, PCT=proximal convoluted tubule, BC=bowman`s capsule, A=control group and B= Sildenafil induced nephrotoxicity group.*

4.3.3 Histosterelogical findings in negative control and positive control groups

The mean glomerulus volume in positive control (sildenafil 1µg/gmbwt/day) group reduced significantly (P=0.0001) as compared to negative control (feeds + water ad libitum) group.

The mean volume of epithelial cells in positive control (sildenafil 1µg/gmbwt/day) group reduced significantly (*P*=0.0001) as compared to negative control (feeds + water ad libitum) group respectively (Table 4.3).

Table 4. 3: Comparative means of absolute glomerulus volume and number weighted volume fraction of epithelial cells in negative and positive control groups

	Control and experimental groups.		
Stereological measurements	**Control (feeds + water ad libitum)**	**SIN (1µg/gmbwt/day sildenafil)**	**P value**
Absolute glomerulus volume(×10^3µm3)	29507.87±.192	22844.53±.256	**0.0001**
Number-weighted volume fraction of proximal tubular cells (epithelial cells)	1.8785±.0002	0.3087±.0002	**0.0001**

KEY: *All values are expressed as the mean± 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*

4.4 Gross histomorphological and stereological changes that occur on kidney on administration of different doses of *Curcuma longa*

4.4.1 Gross morphometric findings of the kidney

4.4.1.1 Mean terminal body weight, mean weight of right and left kidney and mean volume right and left kidney between positive control group and experimental groups

The mean terminal body weight group four (MCL77.5mg/gm/bwt/day) and five (HCL155mg/gm/bwt/day) was statistically significantly different (*P*=0.0001) and (*P*=0.0001) respectively as compared to the positive control (sildenafil 1µg/gmbwt/day) group. The mean weight of right kidney in group four and five was statistically significantly different (*P*=0.0001, *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group whereas the mean weight of left kidney in group four and five was statistically significantly different

(*P*=0.0001, *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group. The mean volume of right kidney in the group four and five was statistically significantly different (P=0.0001, *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group. The mean volume of left kidney in the group four and five was statistically significantly different (P=0.0001, *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group respectively (Table 4.5).

Table 4. 4: The mean terminal body weight, mean kidney weight and volume between positive control group and experimental groups

Mean body weight, kidney weight and volume in gms and mls					
Groups	**Mean terminal body weight**	**Mean weight right kidney**	**Mean weight left kidney**	**Mean volume right kidney**	**Mean volume left kidney**
SIN 1µg/gmbwt/day	254.74±.81	0.94±.02	0.82±.04	1.38 ±.04	1.14 ±.02
Low *Curcuma longa* dose (38.75mg/gm/bwt/day)	270.92±2.23	0.98±.06	0.90±.03	1.39 ±.11	1.24 ±1.59
Medium *Curcuma longa* dose (77.5mg/gm/bwt/day)	285.22±1.76	1.02±.02	0.94±.02	1.66±06	1.58 ±1.61
High *Curcuma longa* dose (155mg/gm/bwt/day)	288.98±2.2	1.18±.11	0.96±.02	1.86±.11	1.82 ±.08

KEY: All values are expressed as the mean± 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 dose, MCL- medium Curcuma longa dose and HCL-high Curcuma longa dose.

4.4.1.2 Mean terminal body weight, mean weight of right and left kidney and mean volume right and left kidney between positive group and restorative group

The mean terminal body weight of rats in restorative group (different doses of *Curcuma longa*) was statistically significantly different (*P*=0.0001) when compared to positive control (Sildenafil 1µg/gmbwt/day) group. The mean weight of right and left kidney in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to positive control (Sildenafil 1µg/gmbwt/day) group. The mean volume of right and left kidney

in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to positive control (Sildenafil Iμg/gmbwt/day) group respectively (Table 4.2).

Table 4. 5: The mean terminal body weight, mean kidney weight and volume between positive control group and restorative group

Groups	Mean body weight, kidney weight and volume in gms and mls Mean terminal body weight	Mean weight right kidney	Mean weight left kidney	Mean volume right kidney	Mean volume left kidney
SIN 1μg/gmbwt/day	254.74±.81	0.94±.02	0.82±.04	1.38 ±.04	1.14 ±.02
Restorative group	281.71 ±2.36	1.66 ±.18	0.93 ±.06	1.88 ±.73	1.42±.08
P value	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

KEY*: All values are expressed as the mean± 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.*

4.4.1.3 Comparative mean of total kidney weight, percentage ratios kidney weights to mean body weight in controls against experimental groups

It was observed that the mean weight percentage ratio of right kidney in positive control group (sildenafil 1μg/gmbwt/day) reduced 0.37% as compared to the negative control group (feeds + water) 0.41% whereas the mean weight percentage ratio of right kidney in high Curcuma longa dose group increased (0.41%) as compared to positive control group (sildenafil 1μg/gmbwt/day) (Table 4.6).

Table 4. 6: A table indicating the total kidney weight and percentage ratios kidney weights to mean body weight in control against experimental groups

Groups	Gross measurements				
	Weight of rats	**Weight of right kidney**	**Weight of left kidney**	**Mean % right kidney weight ratio**	**Mean % left kidney weight ratio**
Control (feeds+ water ab libitum)	284.78±.32	1.16±.08	0.92±.02	0.41%	0.32%
SIN 1µg/gmbwt/day	254.74±.81	0.94±.02	0.82±.04	0.37%	0.32%
Low *curcuma longa* 38.75mg/kg/day	270.92±2.23	0.98±.06	0.90±.03	0.36%	0.33%
Medium *curcuma longa* 77.5mg/kg/day	285.22±1.76	1.02±.02	0.94±.02	0.36%	0.33%
High *curcuma longa* 155mg/kg/day	288.98±2.2	1.18±.11	0.96±.02	0.41%	0.33%

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*

4.4.1.4 Comparative mean width and thickness between positive control and experimental groups

The mean length of right kidney in group 4 and 5 was statistically significant (*P*=0.0001 and *P*=0.0001) as compared to positive control group (Sildenafil1µg/gmbwt/day) whereas the left kidney of group 4 and 5 was statistically significant (*P*=0.0001 and *P*=0.0001) respectively as compared to positive control group (Sildenafil1µg/gmbwt/day) respectively. The mean thickness of right kidney in group 4 and 5 was statistically significant (*P*=0.0001 and *P*=0.0001) respectively when it was compared to positive control group (Sildenafil1µg/gmbwt/day) while the thickness of left kidney was statistically significant (*P*=0.0001 and *P*=0.0001) when in group 4 and 5 as compared to positive control group (Sildenafil1µg/gmbwt/day) respectively (Table 4.7).

Table 4. 7: A table indicating mean length, width and thickness between positive controls and experimental groups

Groups	Kidney gross morphometries in mm.					
	Mean right kidney length	**Mean left kidney length**	**Mean right kidney width**	**Mean left kidney width**	**Mean right kidney thickness**	**Mean left kidney thickness**
SIN 1microgram/gmbwt/day (5 rats)	14.16±.36	11.97±.58	10.27±.16	9.66±.22	3.03±.07	2.91±.02
Low curcuma longa dose (38.75mg/gm/bwt/day)	17.15±.34	16.64±.30	10.18±.70	9.99±.18	4.76±.44	4.18±.11
Medium curcuma longa dose (77.5mg/gm/bwt/day)	17.25±.20	16.75±.15	10.48±.36	10.11±.32	4.78±.23	4.36±.19
High curcuma longa dose (155mg/gm/bwt/day)	17.69±.30	17.46±.23	10.74±.24	10.31±.27	4.90±.27	4.59±.24

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*

4.4.1.5 The mean length, width and thickness between positive control and restorative group

The mean length of right and left kidney in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group. The mean thickness of right and left kidney in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group respectively (Table 4.8).

Table 4. 8: The mean length, width and thickness between positive controls and restorative group

	Kidney gross morphometries in mm.					
Groups	**Mean right kidney length**	**Mean left kidney length**	**Mean right kidney width**	**Mean left kidney width**	**Mean right kidney thickness**	**Mean left kidney thickness**
SIN 1µg/gmbwt/day (5 rats)	14.16±.36	11.97±.58	10.27±.16	9.66±.22	3.03±.07	2.91±.02
Restorative groups (15 rats)	17.37±.17	16.95±.16	10.46±.26	10.14±.14	4.81±.17	4.38±.11
P value	**0.0001**	**0.0001**	1.000	0.264	**0.0001**	**0.0001**

KEY: *All values are expressed as the mean± 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*

4.4.2 Kidney histo-morphological findings among the experimental groups

In low *Curcuma longa* dose*(*38.75mg/kg/day*)*, the glomerulus had defined margins and was surrounded by a bowman`s space. The epithelial cells appeared normal around the cortical tubule while the lumen had defined borders. In medium *Curcuma longa* dose(77.5mg/kg/day), glomerulus had defined margins, narrow bowman`s space, the lumen was well defined (Figure 4.3).

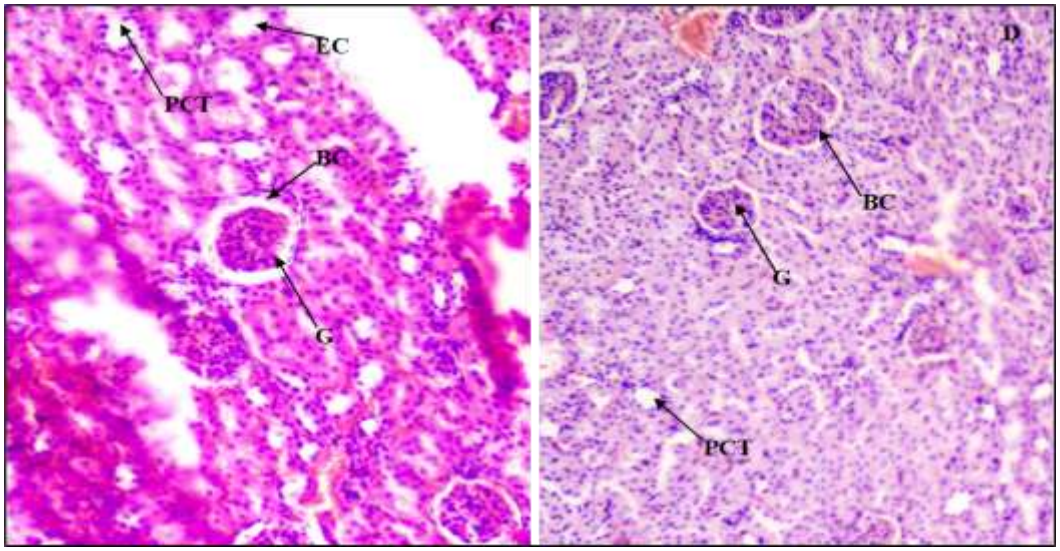

Figure 4. 3: *Photomicrograph C, low curcuma longa dose showing glomerulus with defined margins, normal epithelial cells, bowman`s capsule and proximal convoluted tubule with defined edges. D, in medium curcuma longa dose showing normal glomerulus with well-defined margin*

KEY: *G=glomerulus, PCT=proximal convoluted tubule, BC=bowman`s capsule, EC= epithelial cells, C=low curcuma longa dose group and D= medium curcuma longa dose group.*

In high *Curcuma longa* dose(155mg/kg/day), the glomerulus had well defined margins and a narrow bowman`s space, the epithelial cells appeared normal and cuboidal and the lumen had well defined brush borders (Figure 4.4).

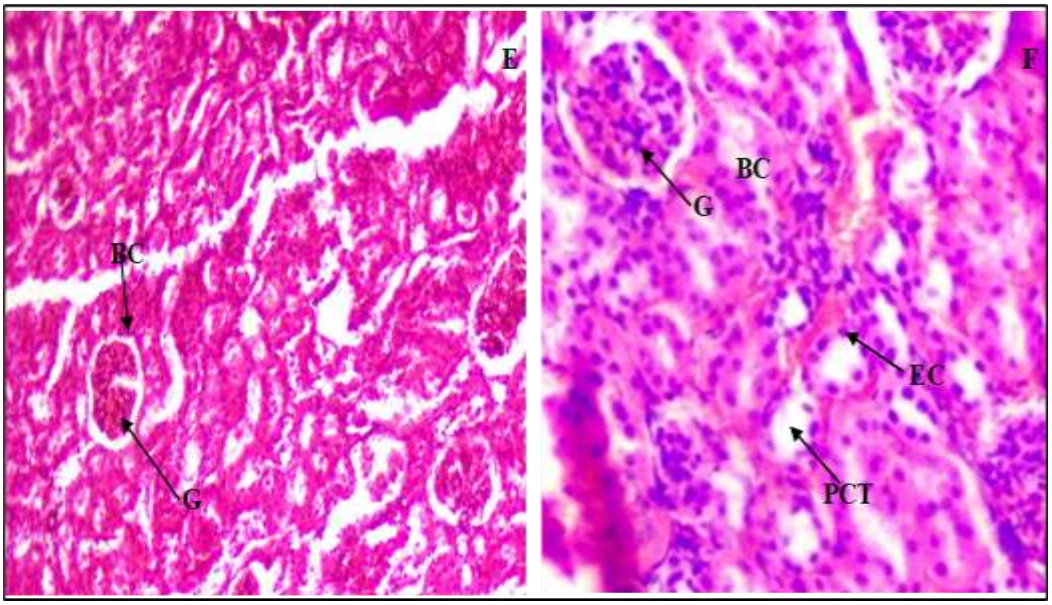

*Figure 4. 4**: Photomicrograph E and F high curcuma longa dose showing normal glomerulus with well-defined margins, narrow bowman`s capsule and cortical tubule normal epithelial cells. At x 10, H&E staining.*

KEY: *G=glomerulus, PCT=proximal convoluted tubule, BC=bowman`s capsule, EC= epithelial cells, E and F=high curcuma longa dose group.*

4.4.3 Kidney Histosterelogical findings among the experimental groups

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

The mean glomerulus volume of medium and high *Curcuma longa* dose group was statistically significantly different (*P*=0.0001) and (*P*=0.0001) respectively as compared to positive control (sildenafil 1µg/gmbwt/day) group. The mean number weighted volume fraction of proximal tubular cells of medium and high *Curcuma longa* dose groups was statistically significantly different (*P*=0.0001) and (*P*=0.0001) respectively (Table 4.8) as compared to positive control (sildenafil 1µg/gmbwt/day) group.

Table 4. 9: Comparative means of absolute glomerulus volume and number weighted volume fraction of epithelial cells in control group and experimental groups

	Control and experimental groups.			
Stereological measurements	**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(×10^3µm3)	22844.53±.256	22544.33±.153	26191.80±.296	28428.20±35.915
Number-weighted volume fraction of proximal tubular cells (epithelial cells)	0.3087±.0002	0.3131±.0053	0.9228±.0001	1.2334±.0001

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*

4.5 To evaluate the renal biochemical parameters of BUN and Creatinine following administration of Curcuma longa on sildenafil induced nephrotoxicity among male albino rats

The table below represents normal values of Blood Urea & Nitrogen and Serum Creatinine levels as adopted from the University of Nairobi Veterinary Laboratory.

Table 4. 10: Normal ranges of biochemical parameters (Blood Urea and Nitrogen and creatinine)

Biochemical parameter	**Normal ranges (mmol/L and mg/dl)**
Blood Urea and Nitrogen	4.2 – 8.97 mmol/L
creatinine	0.2 – 0.8 mg/dl

4.5.1 Comparative renal biochemical marker findings between control groups and experimental groups

Renal parameters were compared between the control groups (negative control and positive control) and experimental groups (restorative groups) using a One Way ANOVA and an inter group significance test done using post hoc Bonferroni. The mean blood urea and nitrogen in positive control(sildenafil1µg/gmbwt/day) increased significantly(*P*=0.0001) as compared to negative control group (feeds+ water). The mean creatinine levels in positive control group (sildenafil1µg/gmbwt/day) increased significantly(*P*=0.0001) as compared to negative control group (feeds+ water) respectively (Table 4:11).

Table 4. 11: Comparative renal biochemical marker findings between negative and positive control groups

	Renal biochemical parameters			
Groups	**Blood urea and nitrogen (BUN) in mmol/L**	**P values**	**Creatinine Mg/dl**	**P values**
Control (feeds+ water ab libitum)	6.6000±.02983	**0.0001**	0.5160±.01077	**0.0001**
SIN 1microgram/gmbwt/day	11.366±.04622	**0.0001**	1.2640±.00510	**0.0001**

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.*

4.5.2 Comparative renal biochemical marker findings between positive control group and experimental groups

There was no change in experimental groups low, medium and high *Curcuma longa* dose as compared to the positive control(sildenafil1µg/gmbwt/day) group.

Table 4. 12: Comparative renal biochemical marker findings between control groups and experimental groups

		Renal biochemical parameters		
Groups	**Blood urea and nitrogen (BUN) in mmol/L**	**P values**	**Creatinine Mg/dl**	**P values**
SIN 1microgram/gmbwt/day	11.366±.04622	**0.0001**	1.2640±.00510	**0.0001**
Low C*urcuma longa* 38.75mg/kg/day	11.248±.01685	0.065	1.2000±.02121	0.069
Medium *Curcuma longa* 77.5mg/kg/day	11.222±.01715	0.014	1.1920±.01772	0.029
High *Curcuma longa* 155mg/kg/day	11.214±.01327	0.009	1.1780±.01497	0.006

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*

CHAPTER FIVE

DISCUSSION

5.1 Gross morphological, histomorphological and histosterelogical changes that occur on kidneys on administration of Sildenafil among male albino rats

In this study the control groups were grouped into negative control (feeds+ water ad libitum) and positive control (SIN 1 microgram/gmbwt sildenafil). During gross anatomical observation after dissection, the two kidneys lay on each side of upper lumbar vertebrae within abdominal cavity, this concurred with the normal anatomical relation of Kidneys as observed by other authors (Olukole, 2021) . The superior anterior part of right kidney related to liver while left was associated with pancreas, stomach, spleen, small intestines and descending colon. These findings were similar to those recorded by (Al-Samawy, 2012) in albino rats.

On examination of borders, the lateral border was convex in shape while the medial border was concave and indented at hilus. On examination of the kidneys of rats that were subjected to sildenafil they appeared darker brown in color, shrinked and pale. The obvious shrinkage may have been due to the increased oxidative stress imposed by free reactive and nitrogen oxide radicals released by sildenafil as was observed by (Kirbas *et al.,* 2015) who recorded the same in the literature on paracetamol effect on kidney and significant reduction in growth of collagen fibers in kidneys as an effect of sildenafil administration and **(**Hegazy *et al.,* 2021**)** on effects of paracetamol on kidney since the two drugs exhibit a similar nephrotoxicity model.

The current study noted a significant (*P*=0.0001) reduction in weight of the rats on treatment with sildenafil as compared to control group, in addition, the mean terminal or live weight of control group was 284.78±.32gm, this was slightly higher as compared with previous studies carried out in (Nigeria)(Onyeanusi *et al*., 2009) of 140.625±3.07gm and similar to (Olukole, 2021) as seen in domesticated African great cane rat. The high weight observed in control

group might have been due to the long duration of study and no stress factors that could interfere with eating habits and weight gain.

The mean weight of rats in SIN group was 254.74±.81gm in the present study, this was also higher as compared to a study done by (Sivasankaran *et al.,* 2007) who noted a mean wight of 183.16±3.76gm after administration of sildenafil**.** Similar trend in reduction of weight of rats when exposed to sildenafil were also noted by (Mohamed Yousry *et al.*, 2016; Ngulde *et al.,* 2016). The reduction in body weight might suggest that the nephrotoxicity process that was taking place as a result of administration of sildenafil. Nephrotoxicity leads to accumulation of toxins in the blood associated with reduced metabolism and loss of appetite, the decrease in production of erythropoietin might also lead to anaemia thus causing fatigue, loss of appetite and finally weight loss. The weight of right kidney was higher than left kidney representing 0.41% and 0.32% respectively which was similar wistar rats and domesticated African great cane rats in the savanna zones of Nigeria (Onyeanusi *et al.*, 2009)**.** However, this was lower than 0.76% results obtained by(Hebel & Stromberg, 1976). This difference could be due to the variations in breed, environmental factors and age.

In the current study, it was observed that sildenafil caused gross glomerular damage and widening of bowman`s space which was mediated by cellular degeneration and necrosis (Figure4.2B). This observation is in agreement with the results reported by (Mohammed & Khudair, 2020) who observed similar changes when diclofenac was used in induction of Acute kidney injury. There was obvious distortion, shrinkage of the glomerulus and glomerulosclerosis (Figure 4.2B) on microscopic study in concurrence with the findings of (Ali *et al.*, 2018)**.** This progressive morphological changes in histoarchitecture of kidney was due to decrease in glomerular filtration of the drug as a result of capillary constriction and increased pro-inflammatory cytokines which lead to a primary renal injury.

There were vacuolations seen in the current study (Figure 4.2) which was mediated by increase in production of lactate that accumulates within the cytoplasm and potentially leading to cellular membrane damage as noted in previous studies (Suriyakumari *et al.,* 2016). There was damage and necrosis of epithelial cells and massive dilatation of proximal tubules and distorted lumen. Previous studies observed that these changes might have been modulated by infiltration of inflammatory cells, intensive swelling of epithelial cells when exposed to oxidant stress, this led to increased production of nitric oxide that reacts with free radicals causing renal damages (Kirbas *et al.,* 2015).

In the current study, the absolute glomerulus volume and mean volume of epithelial cells reduced significantly (P=0.0001) when the negative group was compared with positive control group. This was also noted by (Padmini & Kumar, 2012; Sepehri *et al.,* 2013) in India, on renal effects of gentamicin on kidneys leading to similar nephrotoxic pathway as Sildenafil. This reduction in glomerular volume may have been due to atrophy of glomerulus as a result of infiltration of cytokines and vasoconstriction of glomerular capillaries, thus reduced filtration followed by drug accumulation. The reduction in epithelial volume in PCTs was due to the destruction and necrosis of epithelial cells as a result of increase in generation of nitric oxide that react with free radicals thus causing renal damage to the epithelial cells and lumen of proximal convoluted tubule (Ragab *et al.,* 2022)**.**

5.2 Effects of different doses of *Curcuma longa* on sildenafil induced nephrotoxicity among male albino rats

Curcuma longa is a traditional herbal plant that exhibits both antioxidative and anti-inflammatory benefits (Ghosh *et al.,* 2009)**.** On the contrary, Sildenafil is known to produce free oxidative and nitrogen oxide radicals that cause inflammation by stimulating the release of inflammatory markers. *Curcuma longa* therefore, reacts with these radicals forming a compound that has less effect and able to prevent acute kidney injury. In the current study,

different doses of *Curcuma longa* were used to restore sildenafil induced nephrotoxicity. It was observed that *Curcuma longa* restored the inflicted kidney injuries, improved the histological and morphological structure of kidney (Figure 4.3 & 4.4). (Momenkiaei & Raofie, 2019) made similar observations when he administered 75mg/kg/day *Curcuma longa* as an ameliorative agent, and replicated similar findings by restoring the histomorphological changes on the filtration system. This reconstructed gross histoarchitecture of the kidney may have been due to curcumin that react with nitric oxide released thus reducing the nephrotoxicity progression pathway.

In the current study, it was observed that medium dose of *Curcuma longa* was able to reduce the bowman`s space, restore the glomerular structure and modeling of PCT lumen (Figure 4.3). The previous studies in Mexico of (Tapia *et al.,* 2013) reported similar observations upon use of 120mg/kg/day of *Curcuma longa* when assessing the glomerular hemodynamic changes in rats. The authors report that there was improvement in glomerular blood capillaries supply, filtration rate and drug clearance. This change might have been due to Curcumin which is key component in mitigating tubular necrosis and improving renal function, glomerulus structuring, dilation of glomerular capillaries and increased drug clearance and filtration of the glomerulus.

High *Curcuma longa* dose(155mg/kg/day) proved to be more effective in attaining the restoration and gross morphometrical changes among all the experimental groups (Figures 4.3 & 4.4). This markable restorative features might have been due to the curcumin levels in high *Curcuma longa* dose as it's known to have ameliorative effects on epithelial cells, lumen and glomerular shape. This observation concurs with previous studies (Fan *et al.,* 2013) in Saudi Arabia who used a dose of 200mg/kg/day *Curcuma longa* in assessing the palliative action of Curcumin in gentamicin induced nephrotoxicity. The authors noted a significant improvement in histomorphometry of the kidney and general kidney function.

5.3 Gross morphological, histomorphological and histosterelogical changes on kidneys on administration of *Curcuma longa* in sildenafil induced nephrotoxicity among male albino rats

In the current study, mean weight of experimental groups increased steadily as compared to sildenafil induced nephrotoxicity group. This increase in weight might have been due to Curcumin which is an active component in *Curcuma longa* that serves as a supplementary diet thus improves growth and weight gain rate. Previous studies in Turkey showed similar trends in weight increase whereby rats that were subjected to Curcumin gained more weight as compared to those that were exposed to aflatoxin (Hatipoglu & Keskin, 2022) . The mean weight of right and left kidney significantly (P=0.0001) increased on high *Curcuma longa* dose as compared to positive control group. The noted increased kidney weight might have been due to reduced destruction of renal collagen fibers, reduced inflammatory process and increased kidney histoarchitecture restructuring due to remodeling nature of *Curcuma longa* in nephrotoxicity**.**

The mean volume, length and thickness of kidney in experimental groups increased significantly (P=0.0001) as compared to positive control group. The previous studies observed that these changes might have been due to antioxidant and anti-inflammatory effects of *Curcuma longa* as it helps in improving or protecting the kidney (Akinyemi & Adeniyi, 2018). The increase in volume might have also been due to reduced destruction of epithelial cells by nitric oxide, vasodilation of glomerulus and proximal convoluted tubules as a result of reduced infiltration and increased glomerular filtration rate.

In the current study, there was improvement in general structure of epithelial cells, proximal convoluted tubule had defined margins with a narrow lumen as seen (Figure 4.3 & 4.4) as compared to positive control group. This general improvement in epithelial cells and proximal convoluted tubules may be due to prevention of renal tubular and epithelial cell degeneration

and presence of Curcumin in *Curcuma longa* that restores tubular brush borders and anatomical efficiency of renal tubular basement membrane (Ahmad *et al.,* 2020; Liu *et al.,* 2017).

On observation, current study noted that the glomerulus was histomorphologically restored with well-defined margins after administration of *Curcuma longa* (Figure 4.3 & 4.4) as compared to positive control group (Figure 4.2). (Ahmed *et al.*, 2015) reported a similar result on kidney histomorphology after assessing the effects of Curcumin and garlic on methotrexate and carbon tetrachloride induced nephrotoxicity, they noted a complete histomorphological restoration of glomeruli and its surrounding margins. This markable improvement in restructuring of the glomerulus may have been due to reduced infiltration of inflammatory cells and release of inflammatory markers that prevent necrosis from taking place. Curcumin being an active component in *Curcuma longa* has the ability to restore the glomerular capillaries to normal size thus improving glomerular filtration rate of glomerulus (Russo *et al.,* 2018)**.**

There was increased glomerular volume and mean volume of epithelial cells in experimental groups (Table 4.8). The increase in volume might have been due to restoration of glomerulus, proximal convoluted tubule and epithelial cells to normal. (Ragab *et al.,* 2022) 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.

5.4 Renal biochemical parameters of BUN and Creatinine following administration of *Curcuma longa* on sildenafil induced nephrotoxicity among male albino rats

It was noted that urea increased in positive control group as compared to negative group (Table 4.9). (Liu *et al.*, 2018) observed a similar trend when assessing the biochemical parameter in rats before and after nephrotoxicity. However, (El-Batsh *et al.*, 2021; Wu *et al.*, 2017) recorded reduced levels of urea in rats when assessing the renal biochemical changes. The slight increase in urea levels might have been due to increased production in inflammatory markers and oxidative stress on the glomerulus, proximal convoluted tubule and epithelial cells that cause nephrotoxicity. The contrary observations were noted because of the agents used that had effects on both liver and kidney and this could potentially reduce levels of urea as its can also be synthesized in the liver and kidney.

The levels of urea in experimental group slightly reduced as compared to positive control group (Table 4.9). (Ali *et al.*, 2005) in Brazil demystified similar reports where rats that were exposed to Curcumin or gentamicin + Curcumin showed reduced urea levels signifying positive impact in palliative care. This reduction in urea levels was due to increased anti-inflammatory and antioxidative activities of *Curcuma longa* thus improving the kidney function (Ghosh *et al.*, 2014).

The levels of creatinine in positive control group increased significantly (P=0.001) as compared to negative control group. (Kundu *et al.*, 2012) in Bangladesh noted similar reports while assessing renal function parameters of rat on treatment with ``*Sulvajrini Vatika*`` herb. The increase in creatinine levels were due to impaired renal function, aggregation of necrotic cells within the glomerular capillaries thus reduced filtration rate leading to nephrotoxicity. Studies in Iraq (Kata, 2020) while evaluating the short-time effect of Malathion pesticide on functional changes of kidney in female mice reported a similar trend.

There was no change in urea and creatine levels in experimental groups; low, medium and high *Curcuma longa* dose because nephrotoxicity being a physiological process, then the markers urea and creatinine were still being released into systemic circulation. As the kidney was still undergoing restoration, urea and creatinine were slowly reducing and if the samples were collected many days later the values would have probably reverted to normal ranges. The study therefore, hypothesizes that if the renal biochemical markers (urea and creatinine) were collected several days later then the markers would probably be within the normal ranges.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Summary of key findings

The kidneys that were subjected to sildenafil appeared pale and shrunk. There was a significant increase in terminal body weight of rats in experimental groups as compared to sildenafil induced nephrotoxicity group. There was a consistent increase in mean kidney weight in experimental groups as compared to positive control group. The length, width and thickness steadily increased in experimental groups. The volume as determined by both water immersion method and cavarieli principle increased steadily in experimental groups. The absolute glomerulus volume and number weighted volume of epithelial cells increased consistently in experimental groups as compared to positive control group. Nephrotoxicity in SIN group was characterized by distorted glomerulus, wider bowman`s capsule, necrosis of epithelial cells, wider lumen and dilated proximal tubule with a distorted margin. Restoration in experimental groups was characterized by normal glomerulus, normal epithelial cells, narrow lumen and narrow bowman`s space. Medium and high *Curcuma longa* dose were effective in restoration of histomorphological and stereological defects in the kidney. There were changes in the levels of BUN and Creatinine on administration of *Curcuma longa*.

6.2 Conclusion

It can be concluded that;

1. Sildenafil has effects on kidney histo-morphology and gross morphometry when used in high dose and prolonged period of time. This finding rejects the null hypothesis.
2. *Curcuma longa* restores the gross morphometry and histomorphological structure of kidney in sildenafil induced nephrotoxicity when used as an overdose or prolonged period of time.

3. The study found that *Curcuma longa* has histosterelogical restoration benefits and *Curcuma longa* 77.5mg and 155mg is an efficient dose.
4. The study found that there were changes in the levels of BUN and creatinine when *Curcuma longa* was used to restore sildenafil induced nephrotoxicity. This finding rejects the null hypothesis.

6.3 Recommendations

The study recommends that

1. Sildenafil be used within stipulated doses and time duration.
2. Regulation of sildenafil to reduce adverse effects which leads to acute nephrotoxicity, chronic toxicity and end stage renal disease.
3. *Curcuma longa* can be used as a restorative agent in acute nephrotoxicity as well as chronic kidney toxicity.
4. *Curcuma longa* 77.5 and 155mg/kg has shown to have therapeutic approach to kidney restoration and protection.
5. Further study needs to be conducted to ascertain the pharmacokinetics and pharmacodynamics of effective component of *Curcuma longa* herbal formulation which may lead to restoration of kidney toxicity.

REFERENCES

Adams, L. C., Bressem, K. K., Scheibl, S., Nunninger, M., Gentsch, A., Fahlenkamp, U. L., Eckardt, K.-U., Hamm, B., & Makowski, M. R. (2020). Multiparametric assessment of changes in renal tissue after kidney transplantation with quantitative MR relaxometry and diffusion-tensor imaging at 3 T. *Journal of Clinical Medicine*, *9*(5), 1551.

Ahmad, R. S., Hussain, M. B., Sultan, M. T., Arshad, M. S., Waheed, M., Shariati, M. A., Plygun, S., & Hashempur, M. H. (2020). Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: a mechanistic review. *Evidence-based complementary and alternative medicine*, *2020*.

Ahmed, W., Zaki, A., & Nabil, T. (2015). Prevention of methotrexate-induced nephrotoxicity by concomitantadministration of garlic aqueous extract in rat. *Turkish journal of medical sciences*, *45*(3), 507-516.

Akinyemi, A. J., & Adeniyi, P. A. (2018). Effect of essential oils from ginger (Zingiber officinale) and turmeric (Curcuma longa) rhizomes on some inflammatory biomarkers in cadmium induced neurotoxicity in rats. *Journal of toxicology*, *2018*.

Al-Samawy, E. R. (2012). Morphological and Histological study of the kidneys on the Albino rats. *Al-Anbar J. Vet. Sci*, *5*(1), 115-119.

Ala, M., Mohammad Jafari, R., & Dehpour, A. R. (2021). Sildenafil beyond erectile dysfunction and pulmonary arterial hypertension: Thinking about new indications. *Fundamental & clinical pharmacology*, *35*(2), 235-259.

Ali, B., Al-Wabel, N., Mahmoud, O., Mousa, H., & Hashad, M. (2005). Curcumin has a palliative action on gentamicin-induced nephrotoxicity in rats. *Fundamental & clinical pharmacology*, *19*(4), 473-477.

Ali, B. H., Al Za'abi, M., Adham, S. A., Al Suleimani, Y., Karaca, T., Manoj, P., Al Kalbani, J., Yasin, J., & Nemmar, A. (2018). The effect of sildenafil on rats with adenine—Induced chronic kidney disease. *Biomedicine & pharmacotherapy*, *108*, 391-402.

Aljeboori, K. H., & Majhool, A. (2017). Pathological and Immunological changes induced in male rats treated with therapeutic doses of sustanon. *Al-Anbar J. Vet. Sci*, *10*(1), 52-57.

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.

Bailey, J., Thew, M., & Balls, M. (2014). An analysis of the use of animal models in predicting human toxicology and drug safety. *Alternatives to Laboratory Animals*, *42*(3), 181-199.

Beeton, C., Garcia, A., & Chandy, K. G. (2007). Drawing blood from rats through the saphenous vein and by cardiac puncture. *JoVE (Journal of Visualized Experiments)*(7), e266.

Boolell, M., Gepi-Attee, S., Gingell, J., & Allen, M. (1996). Sildenafil, a novel effective oral therapy for male erectile dysfunction. *British journal of urology*, *78*(2), 257-261.

Borsari, M., Ferrari, E., Grandi, R., & Saladini, M. (2002). Curcuminoids as potential new iron-chelating agents: spectroscopic, polarographic and potentiometric study on their Fe (III) complexing ability. *Inorganica Chimica Acta*, *328*(1), 61-68.

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.

Cavazzini, G. (2019). Archimedes' Principle and the Concept of Gravitation. *Applied Physics Research, 11*(6), 9-18.

Correa, F., Buelna-Chontal, M., Hernández-Reséndiz, S., García-Niño, W. R., Roldán, F. J., Soto, V., Silva-Palacios, A., Amador, A., Pedraza-Chaverrí, J., & Tapia, E. (2013). Curcumin maintains cardiac and mitochondrial function in chronic kidney disease. *Free Radical Biology and Medicine, 61*, 119-129.

Dreesen, E., Bossuyt, P., Mulleman, D., Gils, A., & Pascual-Salcedo, D. (2017). Practical recommendations for the use of therapeutic drug monitoring of biopharmaceuticals in inflammatory diseases. *Clin Pharmacol, 9*, 101-111. https://doi.org/10.2147/CPAA.S138414

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.

El-Batsh, M. M., Samaka, R. M., Elhenawy, E. E. M., Abd Elrahman, A. Y., & Elnaggar, S. R. (2021). Nephroprotective effect of coadministration of curcumin and sildenafil in adenine-induced chronic renal failure in rats. *Menoufia Medical Journal, 34*(1), 297.

Fan, X., Zhang, C., Liu, D.-b., Yan, J., & Liang, H.-p. (2013). The clinical applications of curcumin: current state and the future. *Current pharmaceutical design, 19*(11), 2011-2031.

Fança-Berthon, P., Tenon, M., Bouter-Banon, S. L., Manfré, A., Maudet, C., Dion, A., Chevallier, H., Laval, J., & van Breemen, R. B. (2021). Pharmacokinetics of a single dose of turmeric curcuminoids depends on formulation: results of a human crossover study. *The Journal of Nutrition, 151*(7), 1802-1816.

Garcea, G., Jones, D., Singh, R., Dennison, A., Farmer, P., Sharma, R., Steward, W., Gescher, A., & Berry, D. (2004). Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *British journal of cancer, 90*(5), 1011-1015.

Ghosh, S. S., Gehr, T. W., & Ghosh, S. (2014). Curcumin and chronic kidney disease (CKD): major mode of action through stimulating endogenous intestinal alkaline phosphatase. *Molecules, 19*(12), 20139-20156.

Ghosh, S. S., Massey, H. D., Krieg, R., Fazelbhoy, Z. A., Ghosh, S., Sica, D. A., Fakhry, I., & Gehr, T. W. (2009). Curcumin ameliorates renal failure in 5/6 nephrectomized rats: role of inflammation. *Am J Physiol Renal Physiol, 296*(5), F1146-1157. https://doi.org/10.1152/ajprenal.90732.2008

Giuliano, F., Jackson, G., Montorsi, F., Martin-Morales, A., & Raillard, P. (2010). Safety of sildenafil citrate: review of 67 double-blind placebo-controlled trials and the postmarketing safety database. *Int J Clin Pract, 64*(2), 240-255. https://doi.org/10.1111/j.1742-1241.2009.02254.x

Grossman, E. B., Swan, S. K., Muirhead, G. J., Gaffney, M., Chung, M., Deriesthal, H., Chow, D., & Raij, L. (2004). The pharmacokinetics and hemodynamics of sildenafil citrate in male hemodialysis patients. *Kidney international, 66*(1), 367-374.

Gümüş, B., Vatansever, H. S., Müezzinoğlu, T., Müftüoğlu, S., Kaymaz, F., & Büyüksu, C. (2004). Histopathological effects of sildenafil citrate on rat corpus cavernosum. *Acta histochemica, 106*(1), 37-45.

Hassan, F.-u., Rehman, M. S.-u., Khan, M. S., Ali, M. A., Javed, A., Nawaz, A., & Yang, C. (2019). Curcumin as an alternative epigenetic modulator: mechanism of action and potential effects. *Frontiers in genetics, 10*, 514.

Hatipoglu, D., & Keskin, E. (2022). Ameliorative Effects of Curcumin on Aflatoxin B1-Induced Nephrotoxicity in Wistar-Albino Rats. *Harran Üniversitesi Veteriner Fakültesi Dergisi*, *11*(1), 1-1.

He, L., Peng, X., Zhu, J., Liu, G., Chen, X., Tang, C., Liu, H., Liu, F., & Peng, Y. (2015). Protective effects of curcumin on acute gentamicin-induced nephrotoxicity in rats. *Canadian journal of physiology and pharmacology*, *93*(4), 275-282.

Hebel, R., & Stromberg, M. W. (1976). Anatomy of the laboratory rat. *Anatomy of the laboratory rat.*

Hegazy, A., Abd Al Hameed, E. A., El-Wafaey, D., & Khorshed, O. (2021). Effect of Paracetamol administration on the Rat kidney structure: A Morphological Study. *Zagazig University Medical Journal*, *27*(4), 567-576.

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.

Ilyas, S., Hutahaean, S., & Panjaitan, S. (2019). Effect of Turmeric Rhizome Extract (Curcuma longa L.) on Kidney Histology of Preeclampsia Rats (Rattus norvegicus L.). IOP Conference Series: Earth and Environmental Science,

Kata, F. S. (2020). Short-time effects of malathion pesticide on functional and Histological changes of liver and kidney in female mice. *Pakistan journal of biological sciences: PJBS*, *23*(9), 1103-1112.

Kirbas, A., Cure, M. C., Kalkan, Y., Cure, E., Tumkaya, L., Sahin, O. Z., Yuce, S., Kizilkaya, B., & Pergel, A. (2015). Effect of infliximab on renal injury due to methotrexate in rat. *Iranian journal of kidney diseases*, *9*(3), 221.

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.

Kundu, N. K., Ullah, M. O., Hamid, K., Urmi, K. F., Bulbul, I. J., Khan, M. A. I., Akter, M., & Choudhuri, M. (2012). Studies of lipid profile, liver function and kidney function parameters of rat plasma after chronic administration of sulavajrini vatika. *Pakistan Journal of Biological Sciences*, *15*(14), 666-672.

Kuno, Y., Iyoda, M., Shibata, T., Hirai, Y., & Akizawa, T. (2011). Sildenafil, a phosphodiesterase type 5 inhibitor, attenuates diabetic nephropathy in non-insulin-dependent Otsuka Long-Evans Tokushima Fatty rats. *British journal of pharmacology*, *162*(6), 1389-1400.

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.

Lestari, M., & Indrayanto, G. (2014). Curcumin. Profiles of Drug Substances, Excipients and Related Methodology, 39, 113-204. In.

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

Liu, B., Meng, L., Guan, X., Gao, L., & Trabin, J. (2018). Reversible Acute Kidney Injury Associated with Sildenafil Overdose. *Cureus*, *10*(9).

Liu, F., Ni, W., Zhang, J., Wang, G., Li, F., & Ren, W. (2017). Administration of curcumin protects kidney tubules against renal ischemia-reperfusion injury (RIRI) by modulating nitric oxide (NO) signaling pathway. *Cellular Physiology and Biochemistry*, *44*(1), 401-411.

Lv, J.-C., & Zhang, L.-X. (2019). Prevalence and disease burden of chronic kidney disease. *Renal fibrosis: mechanisms and therapies*, 3-15.

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.

Mehta, R. L., Cerda, J., Burdmann, E. A., Tonelli, M., Garcia-Garcia, G., Jha, V., Susantitaphong, P., Rocco, M., Vanholder, R., Sever, M. S., Cruz, D., Jaber, B., Lameire, N. H., Lombardi, R., Lewington, A., Feehally, J., Finkelstein, F., Levin, N., Pannu, N., . . . Remuzzi, G. (2015). International Society of Nephrology's 0by25 initiative for acute kidney injury (zero preventable deaths by 2025): a human rights case for nephrology. *Lancet*, *385*(9987), 2616-2643. https://doi.org/10.1016/S0140-6736(15)60126-X

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*.

Mohamed Yousry, M., Abas Farag, E., & Ibraheem Omar, A. (2016). Histological study on the potential effect of sildenafil on the kidney and testosterone level in experimentally induced diabetes in male rats. *J Cytol Histol*, *7*, 431.

Mohammed, Y. J., & Khudair, Z. W. (2020). Study the Histopathological Effects of Sildenafil Treatment on the Kidney of Adult Male Rats. □□□□ □□□□□□□ □□□□□□ □□□□□□□□.

Momenkiaei, F., & Raofie, F. (2019). Preparation of Curcuma longa L. extract nanoparticles using supercritical solution expansion. *Journal of Pharmaceutical Sciences*, *108*(4), 1581-1589.

Mshelbwala, F., Waziri, A., & Aji, T. (2019). Histological changes following prolonged oral administration of sildenafil citrate in diabetics rats.

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.

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.

Ngulde, S., Umaru, B., Mahre, M., William, A., Agbutun, P., Midda, Y., Atela, P., Daniel, H., Giwa-Imam, L., & Ngulde, S. (2016). Effect of sildenafil citrate on the body weight, blood glucose and white blood cell count during wound healing process in diabetic rats. *Kanem Journal of Medical Sciences*, *10*(1), 13-20.

Nichols, D. J., Muirhead, G. J., & Harness, J. A. (2002). Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. *British journal of clinical pharmacology*, *53*, 5S-12S.

Olukole, S. G. (2021). Morphometric analysis of the kidneys of the adult domesticated African great cane rat (Thryonomys swinderianus). *European Journal of Anatomy*, *13*(3), 117-120.

Omosa, L., Midiwo, J., & Kuete, V. (2017). Curcuma longa. In *Medicinal spices and vegetables from Africa* (pp. 425-435). Elsevier.

Onyeanusi, B., Adeniyi, A., Ayo, J., Ibe, C., & Onyeanusi, C. (2009). A comparative study on the urinary system of the African Giant Rat (Cricetomys Gambianus Waterhouse) and the Wistar rat. *Pakistan Journal of Nutrition*, *8*(7), 1043-1047.

Padmini, M. P., & Kumar, J. V. (2012). A histopathological study on gentamycin induced nephrotoxicity in experimental albino rats. *IOSR J Dent Med Sci*, *1*(1), 14-17.

Pandya, U., Saini, M. K., Jin, G. F., Awasthi, S., Godley, B. F., & Awasthi, Y. C. (2000). Dietary curcumin prevents ocular toxicity of naphthalene in rats. *Toxicology letters*, *115*(3), 195-204.

Pathak, J., Kharche, S. D., & Goel, A. (2017). Effects of different activation protocols on cleavage rate and blastocyst production of caprine oocytes. *Iran J Vet Res*, *18*(4), 243-248. https://www.ncbi.nlm.nih.gov/pubmed/29387095

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.

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.

Sepehri, G., Derakhshanfar, A., & Saburi, L. (2013). Does propylthiouracil increase the gentamicin-induced nephrotoxicity in rat? *Iranian Journal of Basic Medical Sciences*, *16*(11), 1190.

Sivasankaran, T., Udayakumar, R., Panjamurthy, K., & Singh, V. A. (2007). Impact of sildenafil citrate (Viagra) with ethanol modulates on lipid and lipoprotein in testis of Albino rats. *J Biol Sci*, *7*, 288-293.

Soetikno, V., Watanabe, K., Sari, F. R., Harima, M., Thandavarayan, R. A., Veeraveedu, P. T., Arozal, W., Sukumaran, V., Lakshmanan, A. P., & Arumugam, S. (2011). Curcumin attenuates diabetic nephropathy by inhibiting PKC-α and PKC-β1 activity in streptozotocin-induced type I diabetic rats. *Molecular nutrition & food research*, *55*(11), 1655-1665.

Soni, S. S., Ronco, C., Katz, N., & Cruz, D. N. (2009). Early diagnosis of acute kidney injury: the promise of novel biomarkers. *Blood purification*, *28*(3), 165-174.

Suriyakumari, K., Udayakumar, R., & Ruba, T. (2016). Histological investigations on kidney of sildenafil citrate (edegra) treated albino mice. *Int J Anat Res*, *4*(1), 1977-1980.

Tapia, E., Zatarain-Barrón, Z. L., Hernández-Pando, R., Zarco-Márquez, G., Molina-Jijón, E., Cristóbal-García, M., Santamaría, J., & Pedraza-Chaverri, J. (2013). Curcumin reverses glomerular hemodynamic alterations and oxidant stress in 5/6 nephrectomized rats. *Phytomedicine*, *20*(3-4), 359-366.

Tirkey, N., Kaur, G., Vij, G., & Chopra, K. (2005). Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. *BMC pharmacology*, *5*(1), 1-10.

Ueki, M., Ueno, M., Morishita, J., & Maekawa, N. (2013). Curcumin ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in mice. *Journal of bioscience and bioengineering*, *115*(5), 547-551.

Venkatesan, N., Punithavathi, D., & Arumugam, V. (2000). Curcumin prevents adriamycin nephrotoxicity in rats. *British journal of pharmacology*, *129*(2), 231-234.

Vickers, A. J., Rees, R. W., Zollman, C. E., McCarney, R., Smith, C. M., Ellis, N., Fisher, P., Van Haselen, R., Wonderling, D., & Grieve, R. (2004). Acupuncture of chronic headache disorders in primary care: randomised controlled trial and economic analysis. *Health Technol Assess*, *8*(48), iii, 1-35. https://doi.org/10.3310/hta8480

Wahlström, B., & Blennow, G. (1978). A study on the fate of curcumin in the rat. *Acta pharmacologica et toxicologica*, *43*(2), 86-92.

Wang, J., Re, J., & Wang, Z. (1999). Mode of action of sildenafil. *Zhongguo yi xue ke xue Yuan xue bao. Acta Academiae Medicinae Sinicae*, *21*(6), 493-496.

Wasung, M. E., Chawla, L. S., & Madero, M. (2015). Biomarkers of renal function, which and when? *Clinica chimica acta*, *438*, 350-357.

Witkin, J. M., & Li, X. (2013). Curcumin, an active constiuent of the ancient medicinal herb Curcuma longa L.: some uses and the establishment and biological basis of medical efficacy. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, *12*(4), 487-497.

Wu, J., Pan, X., Fu, H., Zheng, Y., Dai, Y., Yin, Y., Chen, Q., Hao, Q., Bao, D., & Hou, D. (2017). Effect of curcumin on glycerol-induced acute kidney injury in rats. *Scientific reports*, *7*(1), 10114.

APPENDINCES

APPENDIX I:DATA ENTRY FORM

DATA ENTRY FORM ON RESTORATIVE ACTIVITIES OF CURCUMA LONGA ON SILDENAFIL INDUCED NEPHROTOXICITY AMONG MALE ALBINO RATS.

1. **BIOINFORMATION.**

DATE ……...

GROUP CODE ……

ID CODE ……...

GENDER ……...

WEIGHT……...

2. **HISTOMORPHOLOGICAL AND HISTOSTERELOGICAL**
   - **A. GROSS MORPHOLOGICAL**
     1. Weight of the kidney……...
     2. Volume of the kidney…….
     3. Length of the kidney…….
     4. Width of the kidney……...
     5. Thickness of kidney……
   - **B. HISTOSTERELOGICAL**
     1. Glomerulus volume……
     2. Epithelial volume ……...

3.BIOCHEMICAL MARKER LEVELS

1. BUN……

2.Creatinine…….

APPENDIX II:SGS APPROVAL LETTER

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES
Office of the Dean

Our Ref: MSC/SM/00019/2020

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 26th August 2022

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR KHISA WANJALA ALLAN — MSC/SM/00019/020

The above named is registered in the programme of Master of Science in Human Anatomy in the School of Medicine, Maseno University. This is to confirm that his research proposal titled **"Restorative Activities of Curcuma Longa on Sildenafil Induced Nephrotoxicity among Male Albino Rats (*Rattus norvegicus*)"** has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

APPENDIX III:ETHICAL APPROVAL LETTER

OFFICE OF THE CHAIRPERSON
INSTITUTIONAL SCIENTIFIC ETHICS REVIEW COMMITTEE
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B0819012023 January 19, 2023

TO: Khisa Wanjala Allan
Department of Human Anatomy
Maseno University

Dear Allan,

RE: Restorative Activities of Curcuma longa on Sildenafil Induced Nephrotoxicity among Male Albino Rats

This is to inform you that the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/ISERC/08/01/2023. The approval period is 19th January, 2023 – 19th January, 2024.
This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Prof. Jackie K. Obey, PhD
Chairperson, Institutional Scientific Ethics Review Committee

University of Eastern Africa, Baraton
19 JAN 2023
P. O. Box 2500-30100, ELDORET
Research Ethics Committee

APPENDIX IV:NACOSTI REASEARCH LICENSE

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **965904**

Date of Issue: **23/February/2023**

RESEARCH LICENSE

This is to Certify that Mr.. allan wanjala khisa of Maseno University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kisumu on the topic: restorative activities of curcuma longa on sildenafil induced nephrotoxicity among male albino rats. for the period ending : 23/February/2024.

License No: **NACOSTI/P/23/23374**

965904

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013 (Rev. 2014)
Legal Notice No. 108: The Science, Technology and Innovation (Research Licensing) Regulations, 2014

The National Commission for Science, Technology and Innovation, hereafter referred to as the Commission, was the established under the Science, Technology and Innovation Act 2013 (Revised 2014) herein after referred to as the Act. The objective of the Commission shall be to regulate and assure quality in the science, technology and innovation sector and advise the Government in matters related thereto.

CONDITIONS OF THE RESEARCH LICENSE

1. The License is granted subject to provisions of the Constitution of Kenya, the Science, Technology and Innovation Act, and other relevant laws, policies and regulations. Accordingly, the licensee shall adhere to such procedures, standards, code of ethics and guidelines as may be prescribed by regulations made under the Act, or prescribed by provisions of International treaties of which Kenya is a signatory to
2. The research and its related activities as well as outcomes shall be beneficial to the country and shall not in any way;
    i. Endanger national security
    ii. Adversely affect the lives of Kenyans
    iii. Be in contravention of Kenya's international obligations including Biological Weapons Convention (BWC), Comprehensive Nuclear-Test-Ban Treaty Organization (CTBTO), Chemical, Biological, Radiological and Nuclear (CBRN).
    iv. Result in exploitation of intellectual property rights of communities in Kenya
    v. Adversely affect the environment
    vi. Adversely affect the rights of communities
    vii. Endanger public safety and national cohesion
    viii. Plagiarize someone else's work
3. The License is valid for the proposed research, location and specified period.
4. The license any rights thereunder are non-transferable
5. The Commission reserves the right to cancel the research at any time during the research period if in the opinion of the Commission the research is not implemented in conformity with the provisions of the Act or any other written law.
6. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research.
7. Excavation, filming, movement, and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
8. The License does not give authority to transfer research materials.
9. The Commission may monitor and evaluate the licensed research project for the purpose of assessing and evaluating compliance with the conditions of the License.
10. The Licensee shall submit one hard copy, and upload a soft copy of their final report (thesis) onto a platform designated by the Commission within one year of completion of the research.
11. The Commission reserves the right to modify the conditions of the License including cancellation without prior notice.
12. Research, findings and information regarding research systems shall be stored or disseminated, utilized or applied in such a manner as may be prescribed by the Commission from time to time.
13. The Licensee shall disclose to the Commission, the relevant Institutional Scientific and Ethical Review Committee, and the relevant national agencies any inventions and discoveries that are of National strategic importance.
14. The Commission shall have powers to acquire from any person the right in, or to, any scientific innovation, invention or patent of strategic importance to the country.
15. Relevant Institutional Scientific and Ethical Review Committee shall monitor and evaluate the research periodically, and make a report of its findings to the Commission for necessary action.

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