

**Renal histomorphological changes following highly active antiretroviral therapy: possible role of *Hypoxis hemerocallidea* in an experimental animal model**

**By**

**Offor Ugochukwu  
213567926**



**A dissertation submitted to  
Discipline of Clinical Anatomy  
School of Laboratory Medicine and Medical Sciences,  
College of Health Sciences  
University of KwaZulu-Natal  
Durban -South Africa**

**In fulfillment of the Requirement for the Degree of  
Master of Medical Science in Anatomy**

**Supervisor: Dr. O. O. Azu  
Co-Supervisor: Dr. E. C. S. Naidu**

**September, 2015**

## **Preface**

The study described in this dissertation was carried out in the Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa from February 2015 to September 2015, under the supervision of Dr. O.O. Azu and Dr. E.C.S. Naidu.



## **Dedication**

This work is dedicated to my parents Mr. and Mrs. Collins Offor for teaching the child the way he should go and my Uncle Prof. Ozo-mekuri Ndimele.

## Acknowledgements

I am indebted to the following persons for the support they rendered in completion of this project:

I thank my supervisors: Dr. O.O. Azu and Dr. E.C.S. Naidu for their reviews and mentorship in giving me the opportunity to conduct this important research and allowing me to pursue my Masters in Medical Science (M.Med.Sci) under their supervision and also for offering me a shoulder to stand on to see further into the horizon in quest for new knowledge.

I appreciate the support of Jegede Ayoola Isaac who offered constructive suggestion and consultations in helping me organize my dissertation.

I thank my friends and Colleagues in the Discipline of Clinical Anatomy, Nelson R Mandela Medical School, University of KwaZulu-Natal South Africa for their teaming support and encouragement.

My unreserved thanks to all the the Staff of the Biomedical Research Unit (B.R.U) for the use of the facilities in their unit and also for their technical assistance and encouragement.

My profound appreciation to my parents and siblings whose smiles, laughter and prayers are all tonics for the struggle of the Olympian height.

My ultimate gratitude goes to the Supreme Lord, the author of life, for the gift of life itself, for all that has been and has not been and for all that will be and will not be in this life time and into eternity.

**To God, be the glory!**

## Table of contents

	Page
Preface.....	i
Declaration.....	ii
Dedication.....	iii
Acknowledgements.....	iv
Table of contents.....	v-vii
List of figures.....	viii-ix
List of tables.....	x
Abbreviations and Symbols.....	xi-xiii
Abstract.....	xiv-xv

### CHAPTER 1: INTRODUCTION

1.1 Background.....	1-4
1.1.1 Gross anatomy of the kidney.....	4
1.1.1.1 Kidney of other animals.....	6
1.1.1.2 The rat kidney.....	6-7
1.1.2 Embryology of the mammalian kidney.....	7-8
1.1.3 Histology of the kidney.....	10
1.1.4 Functions of the kidney.....	12
1.2 HIV and AIDS.....	14
1.2.1 Modes of transmission of HIV and AIDS.....	14

1.2.2	Global prevalence of HIV and AIDS.....	15
1.2.3	Prevalence of HIV and AIDS in Africa.....	15-16
1.2.4	Management of HIV and AIDS-Antiretroviral therapy.....	16
1.3	Combination of antiretroviral therapy.....	20
1.3.1	HAART-related adverse effects.....	23
1.3.2	HAART-related kidney injuries.....	23-24
1.3.3	Risk factors associated with HAART-related kidney disorder.....	24-25
1.4	Markers of renal injuries.....	25-26
1.4.1	Blood Urea Nitrogen (BUN) .....	26
1.4.2	Serum Creatinine (SCR).....	26
1.4.3	Sodium (Na <sup>+</sup> ).....	26
1.4.4	Potassium (K <sup>+</sup> ).....	27
1.4.5	Chloride (Cl <sup>-</sup> ).....	27
1.5	Interaction of HAART and Plant-based extracts.....	27-28
1.6	<i>Hypoxis hemerocallidea</i> .....	28
1.6.1	Ecology of <i>Hypoxis hemerocallidea</i> plant.....	28-29
1.6.2	Medicinal uses of <i>Hypoxis hemerocallidea</i> plant.....	31
1.6.3	Bioactive components in <i>Hypoxis hemerocallidea</i> corm extract.....	31
1.6.3.1	Hypoxoside and rooperol.....	31-32
1.6.3.2	Phytosterols.....	32-33

1.6.3.3 Lectins.....	33
1.6.4 Pharmacological activities of <i>Hypoxis hemerocallidea</i> extracts.....	34
1.6.4.1 Anti-inflammatory and anti-diabetic activity.....	34
1.6.4.2 Antioxidant activity.....	35
1.6.4.3 Antimicrobial and antiviral activity.....	35-36
1.6.4.4 Cardiovascular and anticonvulsant activity.....	36-37
1.6.4.5 Anticancer activity.....	37-38
1.7 Problem statement.....	38
1.8 Justification for study.....	39-40
1.9 Research questions.....	41
1.10 Objectives of study.....	41
<b>CHAPTER 2: MANUSCRIPT FROM RESEARCH.....</b>	<b>42-57</b>
<b>CHAPTER 3: SYNTHESIS, CONCLUSION AND RECOMMENDATION</b>	
3.1 Synthesis.....	58-60
3.2 Conclusion.....	60
3.3 Recommendation.....	60-61
<b>REFERENCES.....</b>	<b>62-76</b>
<b>APPENDIX</b>	

## List of figures

	Page
Figure 1.1: Schematic diagram showing coronal section of the right kidney.....	5
Figure 1.2: Schematic illustration of the embryology of the mammalian kidney.....	9
Figure 1.3: Cross section of kidney tissue of Sprague-Dawley rats showing various histological structures.....	11
Figure 1.4: Schematic illustration of the structure of the nephron.....	13
Figure 1.5: Photograph showing the African potato plant.....	30
Figure 1.6: Photograph showing the African potato corm.....	30

## Figures from manuscript

Figure 1: Photomicrograph of cross section of kidney of Sprague-Dawley rats Treated with HAART cocktail.....	50
Figure 2: Photomicrograph of cross section of kidney of Sprague-Dawley rats Treated with HAART and low dose of <i>H. hemerocallidea</i> extract.....	50
Figure 3: Photomicrograph of cross section of kidney of Sprague-Dawley rats Treated with HAART and high dose of <i>H. hemerocallidea</i> extract.....	50
Figure 4: Photomicrograph of cross section of kidney of Sprague-Dawley rats Treated with HAART and Vitamin C.....	50
Figure 5: Photomicrograph of cross section of kidney of Sprague-Dawley rats Treated with HAART and Vitamin E.....	51
Figure 6: Photomicrograph of cross section of kidney of Sprague-Dawley rats Treated with HAART and Vitamin (C and E).....	51

Figure 7: Photomicrograph of cross section of kidney of Sprague-Dawley rats  
Treated with low dose of *H. hemerocallidea* extract alone.....51

Figure 8: Photomicrograph of cross section of kidney of Sprague-Dawley rats  
Treated with high dose of *H. hemerocallidea* extract alone.....51

Figure 9: Photomicrograph of cross section of kidney of Sprague-Dawley rats  
In control group.....51

**List of tables**

	Page
Table 1.1: Prevalence of HIV infection and estimated death rates by regions.....	2
Table 1.2: Classification of antiretroviral drugs in use.....	17-19
Table 1.3: Possible combination antiretroviral therapy- FDA approved.....	21-22

**Tables from manuscript**

Table 1: Body weight, kidney weight and KW/BW ratio in groups of rats.....	48
Table 2: Effects of adjuvants of <i>H. hemerocallidea</i> , vitamins C and E With HAART on creatinine and urea levels in the rats.....	49

## **Abbreviations and Symbols**

AIDS	Acquired Immune Deficiency Syndrome
AIN	Acute Interstitial Nephritis
AKI	Acute Kidney Injury
ANOVA	Analysis of variance
AP	African Potato
ART	Antiretroviral Therapy
BPH	Benign Prostate Hyperplasia
BUN	Blood Urea Nitrogen
BW	Body Weight
cART	Combination Antiretroviral Therapy
CD4	Cluster of differentiation 4
CDC	Centre for Disease Control
cDNA	Complementary deoxyribonucleic acid
CKD	Chronic Kidney Disease
CL <sup>-</sup>	Chloride
COX	Cyclooxygenase
CYP3A4	Cytochrome P450 3A4
DNA	Deoxyribonucleic acid
DPX	Permanent mount glue
DU-145	Human prostate cancer cell lines
ECF	Extracellular fluid
FDA	Food and Drug Administration of United States of America
FRAP	Ferric Reducing Ability of Plasma

FS	Fanconi Syndrome
GFR	Glomerular filtration rate
H <sup>+</sup>	Hydrogen ion
HAART	Highly-active Antiretroviral Therapy
H and E	Haematoxylin and Eosin
HIV	Human Immunodeficiency Virus
HIVAN	HIV- associated nephropathy
IC <sub>50</sub>	The half maximal inhibitory concentration
IU	International unit
IUL	Intra-uterine Life
K <sup>+</sup>	Potassium
KBWR	Kidney Body Weight Ratio
KW	Kidney Weight
LDL	Low density lipoproteins
MCF-7	Breast cancer cell line
Na <sup>+</sup>	Sodium
NAD <sup>+</sup>	β- nicotamide adenine dinucleotide
NADH	Reduced β- nicotamide adenine dinucleotide
NASCOP	National AIDS and STI Control Program
NDI	Nephrogenic Diabetes Insipidus
NNRTIs	Non-nucleoside Reverse Transcriptase Inhibitors
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NO	Nitric Oxide
OIs	Opportunistic Infections
PBMCs	Peripheral Blood Mononuclear Cells

PBS	Phosphate buffered solution
PIs	Protease Inhibitors
PLWHAs	People living with HIV and AIDS
PXR	Pregnane X receptor
RFTs	Renal Function Tests
RM	Rotary Microtome
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
SADC	Southern African Development Community
SCR	Serum Creatinine
SD	Standard deviation
T- Lymphocytes cells	Type of white blood cells
THP	Traditional Health Practitioners
TM	Traditional Medicine
UNAIDS	Joint United Nations Program on AIDS
WHO	World Health Organization

## Abstract

### Background:

Nephrotoxicity has become an important public health problem following highly active antiretroviral therapy (HAART), and there is paucity of literature reporting the attenuating influence of plant based adjuvants that can mitigate the effects. The study investigates the role *Hypoxis hemerocallidea* (*H. hemerocallidea*) extract following HAART in an experimental animal model.

### Materials and Method:

Sixty- three adult male Sprague-Dawley rats were used for the study and were divided into 9 groups (A-I). Group A received HAART cocktail (Lamivudine, Stavudine & Nevirapine), Group B received HAART and *H. hemerocallidea* extract (100 mg/kgbw), Group C received HAART and *H. hemerocallidea* extract (200 mg/kgbw), Group D received HAART and vitamin C, Group E received HAART and vitamin E, Group F received HAART, vitamin C and vitamin E, Group G received *H. hemerocallidea* extract (100 mg/kgbw), Group H received *H. hemerocallidea* extract (200 mg/kgbw), and Group I received water as placebo. The experiment lasted for 56 days after which, the animals were sacrificed, the kidneys were harvested and prepared for haematoxylin and eosin (H&E) histological examination and blood samples were collected through cardiac puncture and centrifuged to get the serum for blood urea nitrogen and serum creatinine analyses.

### Results:

Kidney weight changes were not significant except for group A that recorded a significant increase ( $p<0.05$ ) and group B that recorded lowest body weight when compared with the control. Organ-body weight ratios were significantly higher in group A and group F ( $p<0.05$ ).

Adjuvant treatment with *H. hemerocallidea* (in groups B and C) with HAART resulted in increased organ-body ratio, but however not significant. Serum Creatinine (SCR) and blood urea nitrogen (BUN) levels were statistically elevated in HAART-treated animals ( $p<0.05$ , 0.001). SCR levels in group D was significantly reduced ( $p<0.05$ ) but however, significantly elevated in groups B, C, G and H ( $p<0.001$ ). Groups B and C, as well as groups F and H resulted in higher BUN values ( $p<0.05$ ). The histological appearance of group A was highly compromised. When treated concomitantly with *H. hemerocallidea* (at both dosages), no attenuating influence was seen. However, low dose of *H. hemerocallidea* showed improved histological layout as compared to the high dose. Co-administration of HAART and combined dose of vitamin C and E did not improve the histoarchitecture.

**Conclusion**

Adjuvant treatment with *H. hemerocallidea* extract did not attenuate the nephrotoxicity of HAART in this model.

**Keywords:** *kidney, histoarchitecture, Hypoxis hemerocallidea, highly active antiretroviral drugs (HAART).*

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

It is estimated that about 35 million people are living with the human immunodeficiency virus (HIV) worldwide in 2013, and more than 25 million people have died from acquired immune deficiency syndrome (AIDS) since the first cases were reported in 1981. Most of these deaths are due to inadequate access to HIV prevention, care and treatment services (WHO and UNAIDS, 2015). Adolescents aged between 15 to 49 years account for an estimated 12 per cent of HIV infections globally (UNAIDS, 2013).

The toll of HIV/AIDS continues to be harsh in sub-Saharan Africa particularly Southern Africa. In 2013, the region accounted for the vast majority of people living with AIDS. The pandemic killed an estimated 1.5 million people in 2013 of which 1.1 million of the cases were from sub-Saharan Africa (UNAIDS, 2013).

In 2013, UNAIDS estimated that 12% of the South Africa's population of 48 million had HIV/AIDS. The rising prevalence rate increased from 10.6% in 2007 to 12% in 2008, and in 2010, an estimated 280,000 South Africans died of HIV/AIDS (UNAIDS, 2013). The Human Sciences Research Council, a South African institution, estimates that between 42% and 47% of all deaths among South Africans were caused by HIV/AIDS (UNAIDS, 2013). Currently, approximately 6 million South Africans are living with HIV/AIDS with over 2 million on antiretroviral treatment (Shisana *et al.*, 2014). A 2008 study revealed that HIV/AIDS infection in South Africa is distinctly divided along racial lines: 13.6% of black Africans in South Africa are HIV-positive, whereas only 0.3% of whites living in South Africa have the disease (UNAIDS, 2013).

The prevalence of HIV infection and the estimated death rates by regions is shown in table 1.1.

Table 1.1: Prevalence of HIV infection and estimated death rates by regions (for ages 15-49).

<b>World Region</b>	<b>Prevalence of HIV infection</b>	<b>Estimated death rates</b>	<b>Prevalence Rate (%)</b>
Global Total	35.0 million	1.5 million	0.8
Sub-Saharan Africa	24.7 million	1.1 million	4.7
Asia and the Pacific	4.8 million	250000	0.2
Western and Central Europe and North America	2.3 million	27000	0.3
Latin America	1.6 million	47000	0.4
Eastern Europe and Central Asia	1.1 million	53000	0.6
Caribbean	250,000	11000	1.1
Middle East and North Africa	230,000	15000	0.1

Source: World Health Organization (WHO) and United Nations Program on HIV/AIDS (UNAIDS) 2015.

HIV/AIDS has become one of the world's most serious health and development problem necessitating a global effort mounted to address the epidemic, particularly in the last decade. The number of people infected with HIV has also reduced due to created awareness campaigns and introduction of antiretroviral therapy (UNAIDS, 2013).

The introduction of antiretroviral therapy (ART) for the management of HIV and AIDS significantly increased the life expectancy among HIV-infected patients. Between 1996 and 1999 the advent of highly active antiretroviral therapy (HAART) dramatically improved the survival of patients with HIV infection with unprecedented changes in disease progression and mortality seen first in the United States and European populations (Palella, 1998; Pezzotti, 1999). The World Health Organization (WHO) and other organizations are providing countries with ongoing guidance, tools and support in delivering and scaling up ART for HIV/AIDS within the public health sector.

The goal of HAART is to suppress viral replication but its major disadvantage is the adverse side effects accompanying its use. HAART toxicity has emerged as a serious complication and eventually a major reason for possible discontinuation or changes in treatment limiting patient's adherence and therefore, virologic effectiveness (d'Arminio *et al.*, 2000). Acute drug toxicities still exist, and they can affect the quality of life and the patients' willingness to adhere to their treatment regimens (Montessori *et al.*, 2004). This has also led to further push for less toxic, and easy to adhere-to- single tablet monotherapy (Saag and Kilby, 1999).

The most common and troublesome toxicities of HAART includes hepatotoxicity (Abrescia *et al.*, 2005; Soriano *et al.*, 2008) which is linked to mitochondrial damage especially in patients treated with zidovudine, stavudine or didanosine (Walker *et al.*, 2004), anaemia and metabolic disorders especially with protease inhibitors (Sattler *et al.*, 2001), nephrotoxicity especially with nevirapine or tenofovir (Wyatt *et al.*, 2009).

The kidneys have important physiological functions including maintenance of water and electrolyte balance, secretion of hormones and excretion of waste products from the body. In addition, the kidneys also play a role in the excretion of drugs and xenobiotic, and hence may be exposed to high concentrations of drug metabolites making it liable to drug toxicity (Bentley *et al.*, 2010). It is therefore not a surprise that drug-induced renal injury contributes up to 25% of all cases of acute renal failure (Bentley *et al.*, 2010). Drug-induced renal injury may cause cumulative dose-dependent toxicity or idiosyncratic dose-independent toxicity at any time during therapy (Bentley *et*

*al.*, 2010). Renal injury may occur in various renal compartments such as the renal vascular supply, the glomerulus, the collecting ducts, the tubulointerstitium where extensive tubular-peritubular capillary exchange of solutes takes place (Perazella, 2009).

During the process of metabolism, a lot of waste materials are produced in the tissues. Apart from these, the residue of undigested food, drugs, toxic substances and other pathogenic organisms like bacteria are also present in the body. All these substances must be removed to keep the body in a healthy condition. Although various organs in the body are involved in performing these functions, their excretory capacity is limited. The kidney has the maximum capacity of excretory function and so it plays the major role in homeostasis (Sembulingam, 2006).

### **1.1.1 Gross anatomy of the kidney**

The kidneys are bilateral, bean-shaped excretory organs in vertebrates. In humans, the kidneys are located on the posterior abdominal wall in retro-peritoneal position with the right kidney slightly lower than the left due to the asymmetry within the abdominal cavity caused by the liver. The right kidney sits just below the diaphragm and posterior to the liver while the left kidney is below the diaphragm and posterior to the spleen. Each adult kidney weighs between 125 and 170g in males and between 115 and 155g in females. The left kidney is usually slightly larger than the right kidney (Glodny *et al.*, 2009).

Resting on top of each kidney is the adrenal gland. The upper parts of the kidneys are partially protected by the eleventh and twelfth ribs, and each whole kidney and adrenal gland are surrounded by two layers of fat (the perirenal and pararenal fat) as well being covered by the renal fascia (Clapp *et al.*, 2009).

Internally, the kidney is divided into the outer cortex and inner medulla. The medulla further divides into triangular shaped structures called the renal pyramids. The tip of each pyramid empties urine into a minor calyx; minor calyces empty into major calyces, and major calyces empty into the renal pelvis, which becomes the ureter as shown in figure 1.1 below (Clapp *et al.*, 2009).

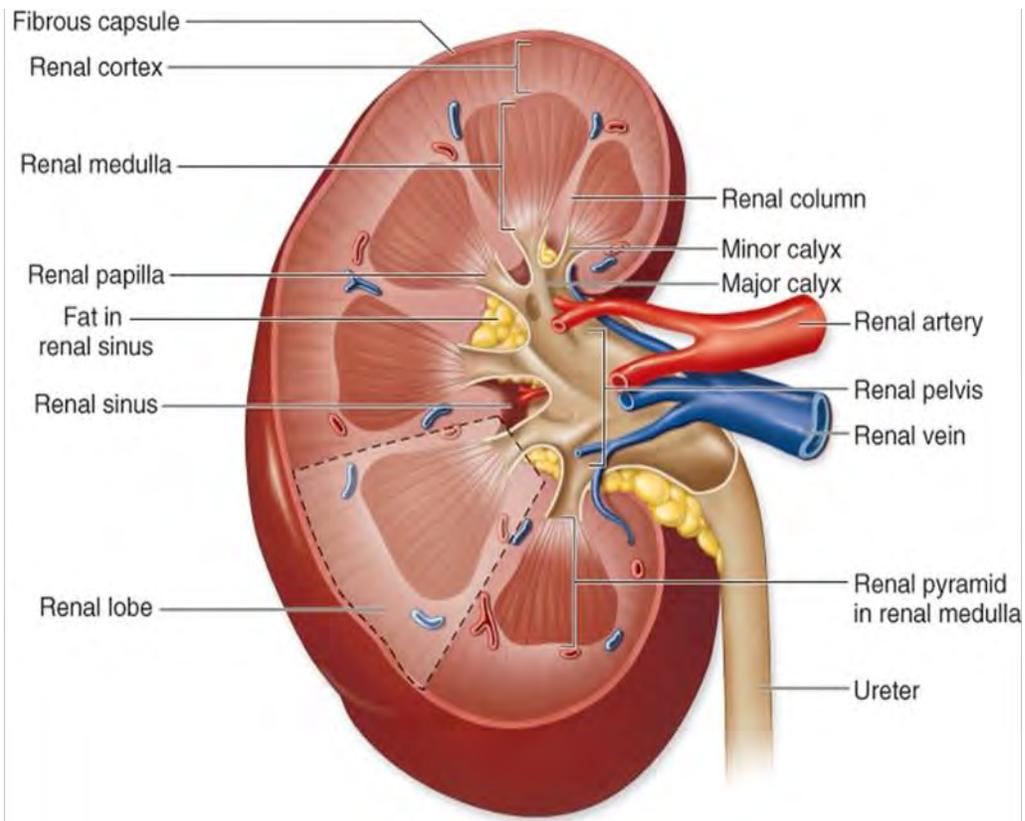


Figure 1.1: Schematic diagram showing coronal section of the right kidney (Adapted from [www. Ivyroses. com/Human Body/.../Urinary\\_System\\_Kidney\\_Diagram](http://www.Ivyroses.com/Human%20Body/.../Urinary_System_Kidney_Diagram)). Assessed on April 22, 2015.

#### **1.1.1.1 Kidney of other animals**

The kidney of fish, amphibians, reptiles, birds and mammals show increasing sophistication in conservation of water and minerals. The kidneys of fish and amphibians are typically narrow, elongated organs, occupying a significant portion of the trunk. The collecting ducts from each cluster of nephrons usually drain into an archinephric duct (Walter, 2004).

The kidneys of reptiles consist of a number of lobules arranged in a broadly linear pattern. Each lobule contains a single branch of the ureter in its center, into which the collecting ducts empty. Reptiles have relatively few nephrons compared with other amniotes of a similar size, possibly because of their lower metabolic rate (Romer *et al.*, 2000).

Birds have relatively large, elongated kidneys, each of which is divided into three or more distinct lobes. The lobes consist of several small, irregularly arranged, lobules, each centered on a branch of the ureter. Birds have small glomeruli, but about twice as many nephrons as similarly sized mammals (Romer *et al.*, 2000).

#### **1.1.1.2 The rat kidney**

The rat kidneys are located ventrolateral to the vertebral column in the retro-peritoneum just like in humans and other mammals (Glodny *et al.*, 2009).

Each rat kidney has a convex and a concave surface. The renal hilum lies in the concave surface and is the point where the renal artery enters the kidney and the renal vein and ureter exit (Bachmann *et al.*, 1998).

Internally, the parenchyma of the rat kidney is divided into two areas: the outer cortex and the inner medulla. The cortex extends into the medulla dividing it into triangular shapes known as renal pyramids. The renal pyramids contain the renal corpuscles and the convoluted tubular segments (Bachmann *et al.*, 1998).

Each adult rat kidney contains roughly 30000-35000 nephrons. The nephron begins in the cortex with the renal corpuscle. The corpuscle consists of a capillary tuft (glomerulus) which is pushed into a blind expansion of the Bowman's capsule. The tubular part of the nephron consists of the proximal convoluted tubule, the loop of Henle and the distal convoluted tubule (Bachmann *et al.*, 1998). According to the location of the corpuscles in the cortex, the nephrons can be distinguished into three types: superficial, midcortical and juxtamedullary nephrons, and according to the length

of the loop of Henle the nephrons of the rat kidney may be subdivided into two different types: nephrons with short loops and nephrons with long loops (Bachmann *et al.*, 1998).

The rat kidney is fairly typical of mammalian kidneys. It is only in mammals that the kidney takes on its classical "kidney" shape, although there are some exceptions, such as the multilobed reticulate kidneys of cetaceans (Romer *et al.*, 2000).

Distinctive features of the mammalian kidney include the presence of the renal pelvis and renal pyramids, and of a clearly distinguishable cortex and medulla as shown in figure 1.1.

### **1.1.2 Embryology of the mammalian kidney**

The mammalian kidney develops from intermediate mesoderm, proceeds through a series of three successive phases, each marked by the development of a more advanced pair of kidneys: the pronephros, mesonephros, and metanephros (see figure 1.2) (Bruce, 2004).

The pronephros develops at the beginning of the fourth week of intrauterine life (IUL) and is represented by 7 to 10 solid cell groups in the cervical region. These groups form vestigial excretory units, called nephrotomes that regress before more caudal ones are formed. By the end of the fourth week of IUL, all indications of the pronephric system have disappeared (Sadler, 2003).

The mesonephros and mesonephric ducts are derived from intermediate mesoderm from upper thoracic to upper lumbar segments. Early in the fourth week of IUL, during regression of the pronephric system, the first excretory tubules of the mesonephros appear. They lengthen rapidly, form an S-shaped loop and acquire a tuft of capillaries that will form a glomerulus at their medial extremity. Around the glomerulus the tubules form Bowman's capsule and together these structures constitute a renal corpuscle. Laterally the tubule enters the longitudinal collecting duct known as the mesonephric or Wolffian duct (Sadler, 2003).

The metanephros or permanent kidney appears in the fifth week of IUL. Its excretory units develop from metanephric mesoderm in the same manner as the mesonephric mesoderm. The collecting duct of the metanephros develops from the ureteric bud, an outgrowth of the mesonephric duct close to its entrance to the cloaca. The bud penetrates the metanephric blastema which is molded over its distal end as a cap. Subsequently the bud dilates forming the primitive renal pelvis and splits into cranial and caudal portions, the future major calyces. Each calyx forms two new buds while penetrating the metanephric tissue. These buds continue to subdivide until 12 or more generations

of tubules have formed. Meanwhile at the periphery more tubules form until the end of the fifth month of IUL. The tubules of the second order enlarge and absorb those of the third and fourth generations forming the minor calyces of renal pelvis. During further development, collecting tubules of the fifth and successive generations elongate and converge on the minor calyx forming the renal pyramid (Sadler, 2003).

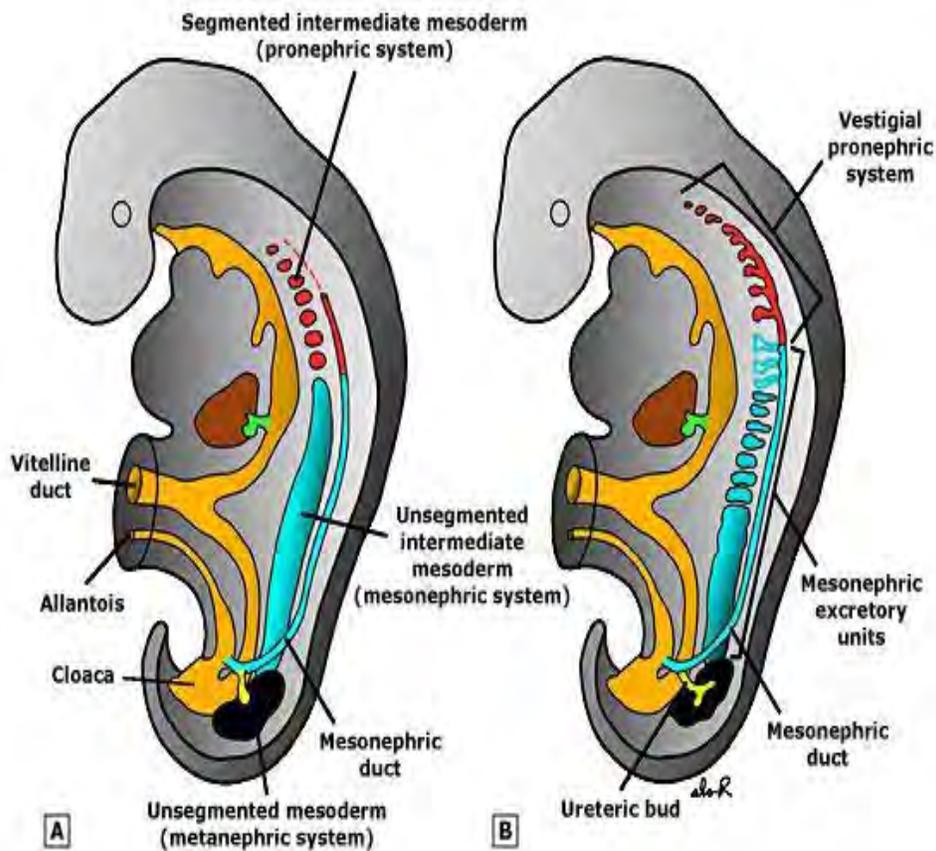


Figure 1.2: Schematic illustration of the embryology of the mammalian kidney (Adapted from Sadler, 2003).

**A.** Relationship of the intermediate mesoderm of the pronephric, mesonephric, and metanephric systems. In cervical and upper thoracic regions intermediate mesoderm is segmented; in lower thoracic, lumbar, and sacral regions it forms a solid, unsegmented mass of tissue, the nephrogenic cord. The longitudinal collecting duct is formed initially by the pronephros but later by the mesonephros.

**B.** Excretory tubules of the pronephric and mesonephric systems in a 5-week old embryo.

### 1.1.3 Histology of the kidney

Microscopic anatomy of the kidney shows that it is composed of interstitial cells and functional units called the nephron. Each nephron consists of tufts of anastomosing capillaries called the glomeruli, formed from the afferent arteriole and draining into the efferent arteriole and a tubular system called the renal tubule. Epithelial cells called podocytes (or visceral epithelium of Bowman's capsule) invest the glomerulus.

The Bowman's capsule is the distended end of the tubular system and is invaginated by the glomerulus (see figure 1.3). The space between the glomerulus and Bowman's capsule is the urinary space. Extending from the capsule is the proximal tubule which is lined by cuboidal and columnar epithelial cells containing many mitochondria and a prominent brush border (Walter, 2004).

The proximal convoluted tubule is the longest portion of the tubular system and is made up of convoluted proximal and distal straight (*pars recta*) segments. The *pars recta* descend into the medulla where it forms the U-shaped loop of Henle. The loop of Henle re-enters the cortex within which it forms the straight and convoluted segments of the distal tubule (see figure 1.4).

The distal tubule runs close to the glomerular hilum and forms a specialized segment called the macula densa. The distal tubule is lined by cuboidal epithelium that lacks a brush border (Walter, 2004). The distal tubule empties into collecting tubules which in turn drain into collecting ducts. The collecting duct converges as they approach the medulla to form the collecting ducts of Bellini which run vertically through the medulla to the papillae. The collecting tubules and ducts are lined by pale-staining, cuboidal epithelial cells (see figure 1.3).

The interstitium is made up of the interstitial cells which consist of fibroblast-like cells, lipid-laden interstitial cells, macrophages, interstitial dendritic cells and perivascular cells. The fibroblast-like cells are mainly located in renal cortex and medulla, and have long cytoplasmic processes forming a reticular network between the tubules and capillaries. In the cortex, the interstitium is small and mainly occupied by small blood vessels. However in the medulla it increases considerably (Al-kahtani *et al.*, 2004).

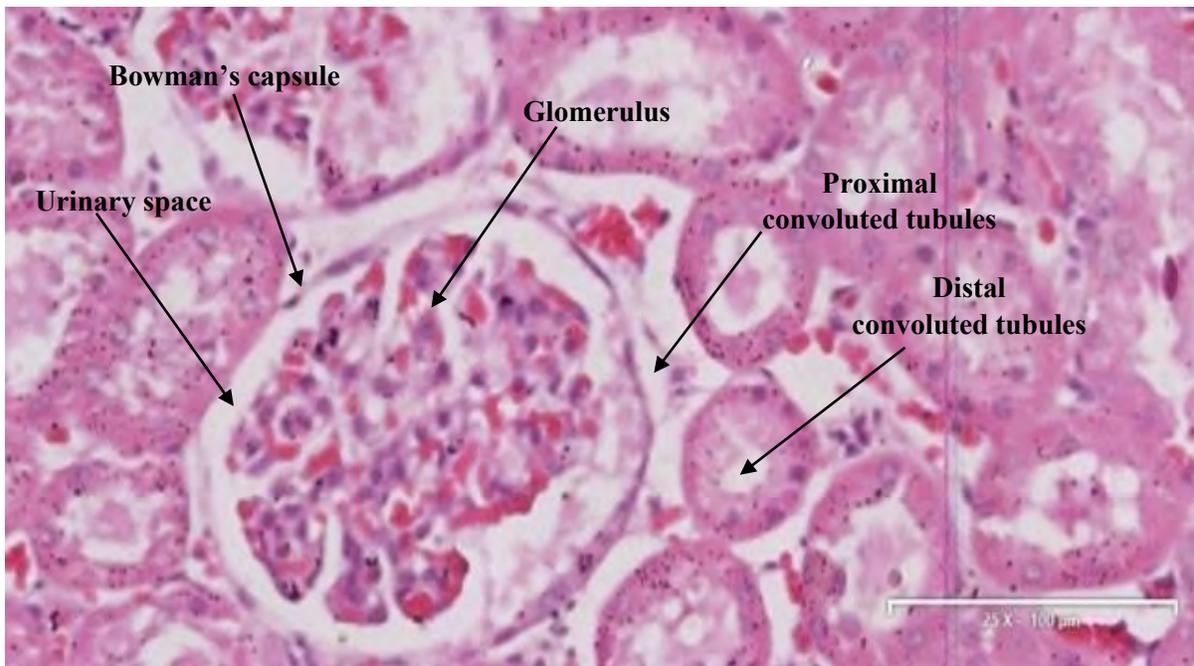


Figure 1.3: Cross section of kidney tissue of Sprague-Dawley rats showing various histological structures. H and E stains.  
(Adapted from Honours research project by Offor U, 2014).

#### **1.1.4 Functions of the kidney**

The kidney performs the function of osmoregulation and excretion of substances through the nephron (see figure 1.4) by the following processes:

1. Filtration of most small molecules from blood to form an ultra-filtrate of plasma.
2. Selective reabsorption of most of the water and some other molecules from the ultra-filtrate, leaving behind excess and waste materials to be excreted.
3. Secretion of some excretory products directly from blood into the urine.
4. Maintenance of acid-base balance by selective secretion of  $H^+$  ions into the urine (Walter, 2004).

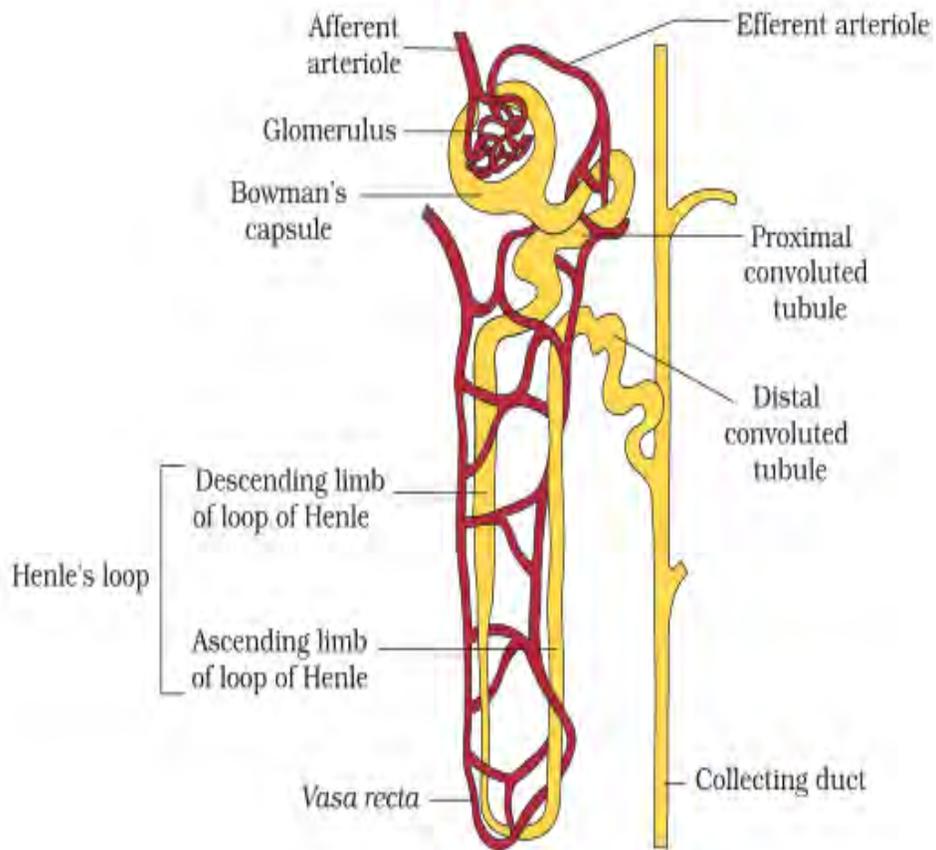


Figure 1.4: Schematic illustration of the structure of the nephron (Adapted from [www. ivyroses. com/Human Body/.../Urinary\\_System\\_Nephron\\_Diagram](http://www.ivyroses.com/Human%20Body/.../Urinary_System_Nephron_Diagram)). Assessed on April 22, 2015.

## **1.2 HIV and AIDS**

HIV belongs to the retrovirus family of viruses. It affects the immune system of infected persons by destroying T-lymphocytes cells, which the body relies to fight infection (NASCOPI, 2002). When HIV enters the body, it infects helper T lymphocytes which display the CD4 receptor binding sites. The virus commandeers the genetic material of the host cell, instructing it to replicate and produce new viral particles which break free from the host, destroying the cell in the process and which then move on to infect and destroy other new uninfected lymphocytes (WHO and UNAIDS, 2015).

There are two distinct serotypes of HIV viruses: type 1 and type 2. The HIV-1 is the primary cause of AIDS worldwide while, HIV-2 is found predominantly in West Africa and its vertical transmission develops more slowly and milder compared to HIV-1 (Sanders *et al.*, 2007).

AIDS is the late stage of HIV infection, a condition characterized by destruction of CD4+ T cells which help the body fight diseases (NASCOPI, 2002). The syndrome was first identified in 1981 among homosexual men and intravenous drug users in New York and California and after its detection evidence of an AIDS epidemic grew shortly after among heterosexual men, women, and children in sub-Saharan Africa (CDC, 2009). Although initial infection with HIV can result in flu-like symptoms, infected persons typically can show no symptoms for many years but as HIV replicate in the body, infected persons begin to show signs and symptoms such as shingles, tuberculosis, oral or vaginal thrush, herpes simplex virus, and Kaposi sarcoma (WHO, 2009) which are a reflection of a weakened immune system or loss of the body's ability to fight infection.

### **1.2.1 Modes of transmission of HIV and AIDS**

HIV is transmitted when a person is exposed to body fluids infected with the virus, such as blood, semen, vaginal secretions, and breast milk. The primary modes of HIV transmission include having unprotected sexual relations with an infected person or sharing hypodermic needles or accidental pricking by a sharp object contaminated with infected blood. It can also be transferred from an infected mother to her baby during childbirth, or through breast-feeding (Milkowski, 2004).

The presence of HIV infection in individuals can be ascertained only through laboratory tests on various body fluids such as blood, plasma, semen or vaginal fluid among others (WHO, 2009). Antibodies to HIV are detectable within four to six weeks of infection by commonly employed tests (Bunel and Cherutich, 2008).

### **1.2.2 Global Prevalence of HIV and AIDS**

HIV/AIDS remains a pandemic and one of the worst to affect humanity over the last century (Oti *et al.*, 2013). Since its discovery in the early 1980s, the infection has quickly developed into a worldwide epidemic, affecting virtually every nation. In 1999, about 26.2 million people were living with the virus. By the end of 2009, it was estimated that 33.3 million people were living with the disease, corresponding to a 27% increase (UNAIDS, 2013). By 2005, more than 20 million people had died of HIV and AIDS (WHO, 2009). Already, more than 25 million people around the world have died of AIDS-related diseases (WHO and UNAIDS, 2015). Globally, about 34% of PLWHAs in 2009 reside in 10 countries in southern Africa; 31% of new HIV infections in the same year occurred in these 10 countries, and about 40% of all adult women with HIV live in southern Africa (UNAIDS, 2013). These are staggering data that impacts negatively on the socio-economic indices in these countries.

### **1.2.3 Prevalence of HIV and AIDS in Africa**

The HIV pandemic has continued to challenge the development and economy of many countries in Africa and has overwhelmed the health-care systems, increased the number of orphans and caused life expectancy rates to reduce. It is one of the single most important health issues threatening the survival of millions of infected people in sub-Saharan Africa (Suave *et al.*, 2002).

Its impact has been most severe in some of the poorest countries in Africa. At the end of 2009, there were 9 countries in Africa where more than one tenth of the adult population aged 15-49 years was infected with HIV (UNAIDS, 2013). For instance, in some countries in the southern part of the continent, including Botswana, Lesotho, Swaziland, Zimbabwe and South Africa, more than 30 percent of the populations have HIV infection or AIDS (CDC, 2009). In Kenya, about 2.3 million people live with HIV/AIDS and each year, approximately 200,000 Kenyans develop the AIDS syndrome (Milkowski, 2004).

HIV/AIDS pandemic affect all regions and communities and it impacts negatively on households and the economic growth of nations. Sub-Saharan Africa is more heavily affected by HIV/AIDS than any other region of the world. In 2008, it was home to two thirds (67%) of all PLWHAs and nearly three quarters (72%) of AIDS-related deaths occurs in this region (WHO and UNAIDS, 2015). It has 'sapped' the population of young men and women in their productive years who form the foundation of the labor force (Ashford, 2006). Health care problems have already reached crisis

proportions in some parts of the world already burdened by war, political upheaval, or unrelenting poverty.

There is increasing evidence that HIV infection of the kidneys is involved with HIV- associated nephropathy (HIVAN) (Winston *et al.*, 1999), with studies suggesting that combination antiretroviral therapy reduces the incidence of HIVAN, possibly by slowing the decline in renal function such that early stages of renal dysfunction are silent and only detectable through laboratory analyses (Szczzech *et al.*, 2004).

#### **1.2.4 Management of HIV and AIDS- Antiretroviral therapy**

Whereas no medical treatment can cure AIDS, ART was developed for the management of HIV and AIDS to reduce viral load and thus improve longevity. The primary goal of ART is suppression of plasma viral load, preservation and restoration of immunologic function, improvement of quality of life and reduction of HIV related morbidity and mortality (NASCO, 2002). Early in the 1980s when the HIV/AIDS epidemic began with no visible medical therapy known to reduce viraemia, people with AIDS were not likely to live longer than a few years (Carcelain *et al.*, 1999). UNAIDS estimated that a total of 2.5 million deaths have been averted in low and middle-income countries since 1995 due to the roll out of antiretroviral therapy (UNAIDS, 2013). In other words, HIV/AIDS is no longer a ‘death penalty’ for victims.

The therapy entails the use of antiretroviral medications that attack the virus itself plus other non-antiretroviral medications to prevent and treat opportunistic infections (OIs) that can occur when the immune system is compromised by the virus (WHO, 2009). Counseling and support mechanisms are also done to help infected people deal with emotional and traumatizing repercussions by encouraging them to accept living with a disabling and potentially fatal disease. This has proven to unlock the declines in transmission of the virus.

ART consists of drugs from five classes; they are nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion or entry inhibitors and integrase inhibitors and are approved by the United States of America Food and Drug Administration (FDA).

The table below (table 1.2) shows the various classes of antiretroviral drugs approved by the FDA.

Table 1.2: Classification of antiretroviral drugs in use.

<b>Drug Class</b>	<b>Mechanism of action</b>	<b>Generic Name (Other names and acronyms)</b>	<b>Brand Name</b>	<b>FDA Approval Date</b>	<b>Manufacturers name</b>
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation.	Abacavir (ABC)	Ziagen	December 17, 1998	GlaxoSmithKline
		Didanosine (ddI, ddI EC)	Videx	October 9, 1991	Bristol Myers-Squibb
			Videx EC (enteric-coated)	October 31, 2000	Bristol Myers-Squibb
		Emtricitabine (FTC)	Emtriva	July 2, 2003	Gilead Sciences
		Lamivudine (3TC)	Epivir	November 17, 1995	GlaxoSmithKline
		Stavudine (d4T)	Zerit	June 24, 1994	Bristol Myers-Squibb
		Tenofovir disoproxil fumarate (TDF)	Viread	October 26, 2001	Gilead Sciences
		Zidovudine (ZDV)	Retrovir	March 19, 1987	GlaxoSmithKline
		Non-Nucleoside Reverse Transcriptase	Inhibit reverse transcriptase directly by binding to the enzyme and	Delavirdine (DLV)	Rescriptor
Efavirenz (EFV)	Sustiva			September	Bristol Myers-

Inhibitors (NNRTIs)	interfering with its function.			17, 1998	Squibb
		Etravirine (ETR)	Intelence	January 18, 2008	Tibotec Therapeutics
		Nevirapine (NVP)	Viramune (Immediate Release)	June 21, 1996	Boehringer Ingelheim
			Viramune XR (Extended Release)	March 25, 2011	Boehringer Ingelheim
		Rilpivirine (RPV)	Edurant	May 20, 2011	Tibotec Therapeutics
Protease Inhibitors (PIs)	Target viral assembly by inhibiting protease enzyme used by HIV to cleave nascent proteins for final assembly of new virions.	Atazanavir (ATV)	Reyataz	June 20, 2003	Bristol-Myers Squibb
		Darunavir (DRV)	Prezista	June 23, 2006	Tibotec, Inc.
		Fosamprenavir (FPV)	Lexiva	October 20, 2003	GlaxoSmithKline
		Indinavir (IDV)	Crixivan	March 13, 1996	Merck
		Nelfinavir (NFV)	Viracept	March 14, 1997	Agouron Pharmaceuticals
		Ritonavir (RTV)	Norvir	March 1, 1996	Abbott Laboratories
		Saquinavir (SQV)	Invirase	December 6, 1995	Hoffmann-La Roche
		Tipranavir	Aptivus	June 22,	Boehringer

		(TPV)		2005	Ingelheim
Fusion or entry Inhibitors	Prevent HIV from binding to or entering human immune cells.	Enfuvirtide (T-20)	Fuzeon	March 13, 2003	Hoffmann-La Roche & Trimeris
		Maraviroc (MVC)	Selzentry	August 6, 2007	Pfizer
Integrase Inhibitors	Inhibit integrase enzyme needed by HIV to insert its genetic material into human cells.	Dolutegravir (DTG)	Tivicay	August 13, 2013	GlaxoSmithKline
		Elvitegravir (EVG)	Vitekta	September 24, 2014	Gilead Sciences
		Raltegravir (RAL)	Isentress	October 12, 2007	Merck & Co., Inc.

**Source:** <https://aidsinfo.nih.gov/education.../fact.../fda-approved-hiv-medicines>

(Last updated 4/28/2015; last reviewed 4/28/2015).

### **1.3 Combination antiretroviral therapy (cART)**

Reports from clinical trials are indicating that monotherapies are not completely effective in combating HIV virulence and this is the basis for cART (Saag and Kilby, 1999).

Recently, the U.S Food and Drug Administration (FDA) approved the combination of 2 or 3 active antiretroviral agents - cART, involving two NRTIs or combined with one medication from either NNRTIs or PIs class (Montessori *et al.*, 2004) as shown in table 1.3.

cART is sometimes referred to as highly active antiretroviral therapy (HAART) and it has become the standard regimen for the management of HIV positive patients (Montessori *et al.*, 2004). It has been effective in suppressing the viral load to undetectable levels resulting in decreased HIV RNA levels and CD4 T cell increases in the vast majority of patients (WHO, 2009). HAART has dramatically decreased the number of hospital admissions and AIDS patients have achieved an impressive improvement in the quality of life (Palella *et al.*, 1998; Soriano *et al.*, 2008). Many people affected by the virus are now living with a manageable chronic condition (Kayode *et al.*, 2011, Kushnir and Lewis, 2011).

Table 1.3: Possible combination antiretroviral therapy- FDA approved.

<b>Drug Class</b>	<b>Generic Name (Other names and acronyms)</b>	<b>Brand Name</b>	<b>FDA Approval Date</b>	<b>Manufacturers name</b>
Multi class combination products	Abacavir and Lamivudine (ABC / 3TC)	Epzicom	August 2, 2004	GlaxoSmithKline
	Abacavir, Dolutegravir, and Lamivudine (ABC / DTG / 3TC)	Triumeq	August 22, 2014	ViiV Health care and Shionogi & Co Ltd.
	Abacavir, Lamivudine, and Zidovudine (ABC / 3TC / ZDV)	Trizivir	November 14, 2000	GlaxoSmithKline
	Atazanavir and Cobicistat (ATV / COBI)	Evotaz	January 29, 2015	Bristol-Myers Squibb
	Darunavir and Cobicistat (DRV / COBI)	Prezcobix	January 29, 2015	Jassen Therapeutics
	Efavirenz, Emtricitabine, and Tenofovir disoproxil fumarate ( EFV / FTC / TDF)	Atripla	July 12, 2006	Bristol-Myers Squibb and Gilead Sciences
	Elvitegravir, Cobicistat,	Stribild	August 27, 2012	Gilead Sciences

	Emtricitabine, and Tenofovir disoproxil fumarate (EVG / COBI / FTC / TDF)			
	Emtricitabine, Rilpivirine, and Tenofovir disoproxil fumarate (FTC / RPV / TDF)	Complera	August 10, 2011	Gilead Sciences
	Emtricitabine and Tenofovir disoproxil fumarate (FTC / TDF)	Truvada	August 2, 2004	Gilead Sciences, Inc.
	Lamivudine and zidovudine (3TC / ZDV)	Combivir	September 27, 1997	GlaxoSmithKline
	Lopinavir and Ritonavir (LPV / RTV)	Kaletra	September 15, 2000	Abbott Laboratories

**Source:** <https://aidsinfo.nih.gov/education.../fact.../fda-approved-hiv-medicines>

(Last updated 4/28/2015; last reviewed 4/28/2015).

### 1.3.1 HAART-related adverse effects

Reduction in mortality and viraemia has been the most outstanding achievement in the global fight against HIV/AIDS in both low and middle income countries (De Visser and Grierson, 2002). Despite these however, there are concerns related to associated toxicities of HAART which have increased (Bodeker *et al.*, 2006).

Increasingly adverse effects caused by HAART ranges from mild to severe and have been well documented in many studies (Hawkins, 2010). Each drug in the HAART combination has its own side effects. The three common side effects of HAART are diarrhea, nausea and fatigue (Emery *et al.*, 2008). Toxicities like lactic acidosis, hepatic steatosis, progressive neuromuscular weakness, dyslipidemia and fat mal-distribution were not recognized until after the drugs had been in use for years (Hawkins, 2010).

HAART side-effects may be transient or may persist throughout therapy and are among the most common reasons for switching or discontinuing therapy (Hawkins, 2010).

Renal dysfunction is a HAART side effect that has been associated primarily with tenofovir disoproxil fumarate (TDF) which actively accumulates in the proximal renal tubule (Cihlar *et al.*, 2001).

### 1.3.2 HAART-related kidney injuries

The kidney plays an important role in the metabolism and excretion of drugs and this makes it liable to various types of injuries such as acute kidney injury (AKI), tubulopathies, chronic kidney disease (CKD) and end-stage renal disease that may require renal replacement therapy (Choi *et al.*, 2009). Clinically, HAART causes various kidney injuries including various electrolyte and acid-base disorders as well as lactic acidosis. These injuries occur via mechanisms such as direct tubular toxicity, allergic reactions and precipitation of insoluble drug crystals within renal tubular lumens (Parazella *et al.*, 2010). As a consequence, the side effects of HAART renal injuries have become a significant cause for concern.

HAART nephrotoxic effects accounted for 14% of late-onset AKI episodes, occurring 3 months after initiating HAART (Roe *et al.*, 2008). In a meta-analysis of 17 studies (including 9 randomized controlled trials) examining TDF safety, a significantly greater loss of kidney function among the TDF recipients compared with control subjects (mean difference in calculated creatinine clearance, 3.92 ml/min; 95% confidence interval, 2.13–5.70 ml/min), as well as a greater AKI risk (risk difference, 0.7%; 95% confidence interval, 0.2–1.2), was noted (Cooper *et al.*, 2010) was reported.

Like most toxic drugs, oxidative stress mediates most pathophysiological processes especially with the use of antiretroviral drugs. The reactive oxygen species (ROS) produced directly act on cell components including lipids, proteins and DNA and destroy their structure. The consequence of this is oxidative stress and cell death (Azu, 2012). It is hypothesized that this toxicity is the result of mitochondrial DNA depletion or direct tubular cytotoxicity similar to that associated with the use of adefovir and cidofovir (Gallant and Deresinski 2003; Cihlar *et al.*, 2001).

### **1.3.3 Risk factors associated with HAART-related kidney disorder**

Risk factors for kidney disease like hypertension, diabetes mellitus, and other related diseases remain significant concerns in HIV patients on HAART (Campbell *et al.*, 2009; Overton *et al.*, 2009). A number of observational studies have documented TDF associated nephrotoxicity following its wide spread use in patients with multiple comorbid conditions (Reid *et al.*, 2008). TDF-induced renal toxicity is more likely to occur in HIV patients with preexisting kidney disease or poorly controlled HIV disease with longer overall antiviral treatment duration, older patients, elevated baseline creatinine concentration, and concomitant administration of other nephrotoxic drugs (Crum *et al.*, 2010; Nelson *et al.*, 2008). Combined therapy with TDF and protease inhibitors such as ritonavir appears to increase renal toxicity (Zimmermann *et al.*, 2006).

Conversely, HAART may increase the risk of hypertension, diabetes mellitus, and other metabolic complications creating a vicious cycle. The possibility of rhabdomyolysis with pigment-related kidney injury should be considered in patients with HIV who develop AKI, particularly if they are being treated with zidovudine, didanosine, or integrase inhibitors (Joshi and Liu 2000; Zembower *et al.*, 2008; Dori *et al.*, 2010).

In a study by Wyatt *et al.*, (2006), the major risk factors for AKI and associated mortality included severe immunosuppression (CD4 count, <200 cells/mm<sup>3</sup>) and opportunistic infections. Dehydration, alkaline urine, and a previous history of nephrolithiasis appear to be risk factors for atazanavir associated kidney stones (Couzigou *et al.*, 2007). The risk factors for hyperlactemia (lactate > 2mmol/l with or without acidosis) which is common with the drugs like stavudine (d4T) and didanosine (ddI) include hypertriglyceridemia, obesity, hepatitis infection, impaired kidney function (Murphy *et al.*, 2003; Bonnet *et al.*, 2005). Ritonavir-boosted PIs may have an increased propensity of causing renal injury. Approximately 70% of the published cases of TDF-induced nephrotoxic effects are observed with concomitant use of low-dose ritonavir. An interaction between lopinavir-

ritonavir combination therapy and TDF, which manifests as a decrease in the renal clearance of TDF, has been identified (Gupta, 2008).

TDF is actively taken up into the proximal tubules and secreted into the lumen via multidrug resistance-associated protein-4 (Ray *et al.*, 2006). Exposure to TDF showed adverse serious renal events and graded elevations in serum creatinine of patients, and it has also been linked to the development of proximal tubular dysfunction including Fanconi syndrome (FS), AKI, nephrogenic diabetes insipidus (NDI), and severe hypokalemia (Gupta, 2008; Labarga *et al.*, 2009).

Lamivudine, stavudine, abacavir, and didanosine have also been implicated in case reports of FS and NDI (Ahmad *et al.*, 2006). FS caused by tenofovir-induced nephrotoxicity is characterized by generalized proximal tubular dysfunction resulting in one or more of the following: bicarbonaturia, glucosuria (with normal blood sugar), phosphaturia, uricosuria, aminoaciduria, and tubular proteinuria (Labarga *et al.*, 2009).

A recent study by the Columbia University group demonstrated that TDF nephrotoxicity is manifested as toxic acute tubular necrosis targeting proximal tubules (including FS in some cases) and manifests in distinctive light microscopic and ultra-structural features of mitochondrial injury (Herlitz *et al.*, 2010). Acute interstitial nephritis (AIN) has been described with indinavir, abacavir, ritonavir, and atazanavir (Chugh *et al.*, 1997; Krishnan *et al.*, 2000). Abacavir causes renal toxicity as part of the systemic clinical syndrome of abacavir hypersensitivity (Mallal *et al.*, 2008).

#### **1.4 Markers of renal injuries**

Renal damage manifests itself as tubular injury with associated reduction in glomerular filtration and patients often develop increased serum creatinine, glycosuria, tubular proteinuria, and low serum phosphate (Thompson, 2011).

Diagnosis of HAART induced adverse effects on the kidney involve performing full renal function tests (RFTs), a group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's kidney (Daugas *et al.*, 2005). Indirect renal markers namely serum creatinine (SCR) and blood urea nitrogen (BUN), as well as electrolytes; sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>) and chloride (Cl<sup>-</sup>) are assayed to determine renal function. Serum creatinine levels or creatinine clearance/glomerular filtration rate can be measured to determine degree of renal dysfunction.

The functional importance of renal biochemical analytes and their levels are significant in diagnosing renal-toxicity. These analytes are summarized below.

#### **1.4.1 Blood Urea Nitrogen (BUN)**

BUN test measures the amount of nitrogen in the blood that comes from the waste product urea.

Urea is the major end product of protein metabolism. It is synthesized by the urea cycle in the liver from ammonia produced by amino acid deamination. Urea is excreted mostly by the kidneys but minimal amounts are also excreted in sweat and degraded in the intestines by bacterial action. Determination of blood urea nitrogen is the most widely used screening test for renal function (Friedman and Young, 2000). It is used in conjunction with serum creatinine determinations to aid diagnosis.

Elevations in BUN concentration indicates that the kidneys are not working efficiently and occurs in some conditions such as inadequate renal perfusion, chronic nephritis, tubular necrosis, glomerulo-nephritis and urinary tract obstruction (Friedman and Young, 2000).

#### **1.4.2 Serum Creatinine (SCR)**

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2% of the body's creatine is converted to creatinine every day (Rock *et al.*, 1986). Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose of it in the urine. The kidneys maintain the blood creatinine in a normal range (0.6-1.3 mg/dl). Creatinine has been found to be a fairly reliable indicator of kidney function (Reid *et al.*, 2008). Elevated creatinine level signifies impaired kidney function or kidney disease. However, in the early stages of kidney damage, a rise in the serum urea levels usually precedes an increase in serum creatinine (Reid *et al.*, 2008).

#### **1.4.3 Sodium (Na<sup>+</sup>)**

Sodium is the major extracellular cation and functions in maintaining fluid distribution and osmotic pressure. Hyponatremia can be caused by prolonged vomiting or diarrhea, diminished reabsorption in the kidney and excessive fluid retention whereas increased sodium level can be caused by excessive fluid loss, high salt intake, and increased kidney reabsorption (Toffaletti and Jones, 1984).

#### **1.4.4 Potassium (K<sup>+</sup>)**

Potassium is the major intracellular cation and is critical to neuromuscular cell activity. Some causes of decreased K<sup>+</sup> levels include reduced dietary intake or excessive loss from the body due to prolonged vomiting, diarrhea or increased kidney excretion. Increase K<sup>+</sup> levels may be caused by dehydration or shock, diabetic ketoacidosis, and potassium retention by the kidneys (Toffaletti and Jones, 1984).

#### **1.4.5 Chloride (Cl<sup>-</sup>)**

Chloride is the major extracellular anion and serves to regulate the balance of extracellular fluid distribution. Common causes of decreased chloride include reduced dietary intake, prolonged vomiting, reduced renal reabsorption, as well as some forms of acidosis and alkalosis. Increased Cl<sup>-</sup> values are found in dehydration, kidney failure, some forms of acidosis, high dietary or parental Cl<sup>-</sup> intake, and salicylate poisoning (Toffaletti and Jones, 1984).

As PLWHA continue to rely on HAART to enhance their quality of life, HAART-related toxicities keeps occurring and are encountered by clinicians (Campbell *et al.*, 2009) and there is need to tackle this challenge.

### **1.5 Interaction of HAART and Plant-based extracts**

Recently, there has been interest in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases (Aruoma *et al.*, 2006), and previous studies (Azu *et al.*, 2010) support the use of plant-based adjuvants for disease management like diabetes, cancers, *etc.* An estimated 80% of Africans are said to rely on complementary and alternative medicines for the management of various pathological conditions (Shah, 2004). For the majority of this group of individuals, consultation with traditional health practitioners (THP) is the first call for health services (Owen-Smith, 2007).

The high burden of toxicities associated with HAART has attracted various studies designed to accommodate a holistic management approach including the use of traditional medicines. Thus, while the use of medicinal herbs, a principal component of traditional medicine, predates the emergence of HAART toxicity, there remains a widespread consumption of herbal products for the management of diseases either as complementary or alternative medicines (Peltzer *et al.*, 2011).

Of the various classes of plants, interest has been focused on medicinal plants with pharmacological properties and the *Hypoxis hemerocallidea* plant has been recognized to possess a wide range of pharmacological properties such as: anti-inflammatory, antioxidant, antimicrobial, anticonvulsant and anticancer properties (Ojewole, 2006; Steenkamp *et al.*, 2006; Ncube *et al.*, 2012).

## **1.6 *Hypoxis hemerocallidea***

*Hypoxis hemerocallidea* (*H. hemerocallidea*) Fisch. & C.A. Mey popularly called African potato belongs to the genus *Hypoxis*, from the large lily family *Hypoxidaceae* (Boukes and van de Venter, 2011). It is one of the most popular herbal remedies consumed among people living with HIV/AIDS in South Africa to boost immunity and enhance general well-being (Boukes and van de Venter, 2012). It is a tuberous, perennial herb with long, strap-shaped leaves and yellow, star-shaped flowers as shown in figure 1.5 and 1.6 respectively.

The broad and slightly hairy leaves of *H. hemerocallidea* are arranged one above the other to form three distinct groups of leaves spreading outwards from the center of the plant, while the bright yellow, star-shaped flowers are borne on long, slender stalks (Musabayane *et al.*, 2005).

The tuberous rootstock (corm) of the *H. hemerocallidea* is referred to in various local languages as Afrika patat (Afrikaans), inkomfe (Zulu) and ilabatheka (Xhosa) (Ojewole *et al.*, 2009). And is widely used in southern African traditional medicine as a remedy for an array of human ailments (Owira and Ojewole, 2009).

### **1.6.1 Ecology of *Hypoxis hemerocallidea* plant**

Taxonomically, the *H. hemerocallidea* plant falls under the star-lily family or *Hypoxidaceae*, which consists of 8 general and 130 species; with 90 of them belonging to Southern Africa (Drewes *et al.*, 2008).

This family usually consists of monocotyledonous plants, which are normally found in the savanna regions of South Africa, Swaziland, Lesotho, Botswana, Mozambique, Zimbabwe and in North-Eastern Africa (Drewes *et al.*, 2008; Katerere and Eloff, 2008). In South Africa, *H. hemerocallidea* plant is found growing in the wild areas of the Eastern Cape, KwaZulu-Natal, Gauteng and Limpopo provinces. It can also be found in the mountainous areas of South America, Australia, and in the coasts of Asia (Drewes *et al.*, 2008).

The *H. hemerocallidea* plant has been described as a stemless, geophytic, perennial herb with large dark brown to black corms (tubers) and bright yellow flowers; the plant is a herbaceous and a

tuberous perennial plant that consists of yellow star-shaped flowers, long strap-like leaves (30 cm long and 3.2 cm wide), brown tuberous rhizomes or corms (up to 10 cm in diameter or length and about a half a kilogram in weight) and lots of adventitious roots that allow them to survive unfavorable conditions as shown in the figures below (Ndong *et al.*, 2006).



*Hypoxis hemerocallidea* leaves grow back in spring, and summer. Its leaves are strap-like and grow out of false sheath like stem which extends downwards into the underground corm.

Six-petal star shaped flower hence the name 'yellow star'. The ovary, inferior to the flower, is not visible.

Figure 1.5: Photograph showing the *H. hemerocallidea* plant (Adapted from Drewes *et al.*, 2008).



The false stem encloses the leaves in a tubular, membranous sheath made from the remnants of old leaves.

Numerous, adventitious roots grow in concentric circles around the upper part of the rhizome.

The mature vertical rhizome grows to 7-10 cm diameters. It is a swollen, underground stem, whose cut surface is yellow-orange in colour which turns brown with oxidation. When the leaves die away in winter, the rhizome is able to survive the harsh freezing conditions. This tuber-like structure must have developed as a revolutionary storage organ to survive high stress conditions.

Figure 1.6: Photograph showing the *H. hemerocallidea* corm (Adapted from Drewes *et al.*, 2008).

## **1.6.2 Medicinal uses of *Hypoxis hemerocallidea* Plant**

The *H. hemerocallidea* plant has been used in South Africa for medicinal purposes, which includes its use as an immune booster in HIV/AIDS treatment (SADC, 2002; Mills *et al.*, 2005). Recently, it is also used in most countries world-wide as a medicinal remedy for malignancies and inflammatory conditions (Drewes *et al.*, 2008; van Wyk, 2008). The plant has long been used by traditional healers to treat a variety of general ailments such as cardiac diseases, impotency and infertility, hypertension (Drewes *et al.*, 2008; Katerere and Eloff, 2008). It has been used to also treat diseases such as diabetes, bronchitis, sore throat, yuppie flu, chronic viral diseases, haemorrhoids, herpes simplex, tuberculosis, and stomach ulcers (Steenkamp *et al.*, 2006; van Wyk 2008; Ojewole *et al.*, 2009).

The plant is now used to treat diverse types of cancers and tumors such as the urinary diseases, testicular tumors, prostatitis, prostate hypertrophy and benign prostate hyperplasia (BPH) (Abegaz *et al.*, 1999; Nair and Kanfer, 2006; Drewes *et al.*, 2008). The plant is also used as a CD4 lymphocytes stabilizer in cancer and HIV/AIDS patients and as a stimulant of muscular and hormonal activities (Mills *et al.*, 2005). Traditionally, *H. hemerocallidea* is prepared by being cut into small cubes which are then boiled for 20 minutes and orally consumed (Nair and Kanfer, 2006).

## **1.6.3 Bioactive components in *Hypoxis hemerocallidea* corm extract**

### **1.6.3.1 Hypoxoside and rooperol**

Studies have been done on the *Hypoxis hemerocallidea* Fisch. & C.A. Mey plant extract by several authors; Albrecht *et al.*, (1995) showed that the extracts contained a yellow water-soluble crystalline compound named hypoxoside. Hypoxoside is the trivial name for (E)-1, 5-bis-(4'- $\beta$ -D-glucopyranosyloxy-3'-hydroxyphenyl) pent-4-en-1-yne, which is a norlingan diglucoside isolated from the corms of the *Hypoxis* plants (Albrecht *et al.*, 1995; Nair and Kanfer 2006).

When hypoxoxide is exposed to  $\beta$ -glucosidase containing media, it is converted and deconjugated into a lipophilic bioactive dicatechol aglucone (rooperol). The enzyme  $\beta$ -glucosidase is mainly found in the human gastrointestinal tract (mainly in the large intestines) (Boukes *et al.*, 2008).

The rooperol compound has been proven to exert growth inhibition properties in 60 human cancer cell lines, and these include the breast, colon, uterus, melanoma and non-small cell lung cancer cell lines (Nair and Kanfer, 2006; Boukes *et al.*, 2008). Albrecht *et al.*, (1995), also showed that

rooperol may play a role in the mechanisms involved in the maintenance of chromosome structural integrity and segregation during mitosis.

*In vivo* studies have shown that upon oral ingestion of the plant extract from the corm, the non-toxic hypoxide is converted by  $\beta$ -glucuronidase (present in the gastro intestinal system) to form rooperol phase 2 metabolites (glucuronidase / sulphates) which are also non-toxic (Smith *et al.*, 1995). Research has further shown rooperol to contain antioxidant (radical-scavenging) capabilities (Laporta *et al.*, 2007), anti-inflammatory (anti COX-1 and anti COX-2 activities) (Jager *et al.*, 1996; Ojewole, 2006; Steenkamp *et al.*, 2006), anticonvulsant (Ojewole, 2002; Mahomed and Ojewole, 2003), anticancer (Drewes *et al.*, 2008), cardio depressant and hypotensive (Ojewole *et al.*, 2006), and bronchorelaxant properties (Ojewole *et al.*, 2009).

### 1.6.3.2 Phytosterols

In addition to hypoxoside and rooperol, *H. hemerocallidea* Fisch. & C.A. Mey plants also possess plant sterols known as phytosterols, which also contribute to the medicinal and therapeutic properties of the plant (Boukes *et al.*, 2008). Phytosterols have a structure that closely resembles that of the cholesterol molecule. Like cholesterol, phytosterols are mainly found in plant cell membranes as membrane stabilizers (Awad and Fink, 2000; Boukes *et al.*, 2008). The most commonly known phytosterols are betasitosterol ( $C_{29}H_{50}O$ ), campesterol ( $C_{28}H_{48}O$ ) and stigmasterol ( $C_{29}H_{48}O$ ), which are only produced in plants. Phytosterols are therefore only obtained by humans through diet, especially from sources like unrefined plant cells, seeds, nuts, legumes, cereals, fruit and vegetables (Awad and Fink, 2000; Boukes *et al.*, 2008).

Experimental studies have shown that phytosterols, particularly  $\beta$ -sitosterol, have anticancer activities against several cancer cell lines such as the colon, prostate and breast cancers (Boukes *et al.*, 2008). This anticancer property of phytosterols is obtained through multiple mechanisms. The protection from cancer by the phytosterols is achieved by the ability of  $\beta$ -sitosterol to affect the membrane integral structure and fluidity mainly by altering the phospholipid concentration, the cholesterol / phospholipid ratio and the fatty acid composition of cancer cells (Awad and Fink, 2000).

Moreover,  $\beta$ -sitosterol stimulates the proliferation of the human peripheral blood lymphocyte (human T-cells) (Bouic *et al.*, 1996) and has no effect on estrogen receptors (Awad and Fink, 2000); thus  $\beta$ -sitosterol can be used as an immune system booster and also for the management of testicular tumors and benign prostatic hyperplasia (BPH) (Wilt *et al.*, 2011).  $\beta$ -sitosterol also has an effect on

membrane-bound enzymes as well as on the signal transduction pathways. Beta-sitosterol stimulates apoptosis in cancer cells by inhibiting cancer cell proliferation and tumor growth (Awad and Fink, 2000). The *Hypoxis* plant contains a combination of  $\beta$ -sitosterol and its glucoside,  $\beta$ -sitosterol glucoside. These compounds are known to treat prostate hypertrophy due to their ability to inhibit the  $5\alpha$ -reductase and aromatase enzymes in order to inactivate the binding of dihydrotestosterone within the prostate gland (van Wyk, 2008).

In some countries, these phytosterols (because of their capability of lowering both the plasma-cholesterol and low density lipoprotein (LDL)-cholesterol levels) are now used to treat mild hypercholesterolaemia and cardiovascular diseases, pulmonary tuberculosis, HIV and immune system suppression (Pegel, 1997). Other important therapeutic properties of phytosterols include them being used as anti-diabetic and anti-inflammatory agents (Pegel, 1997).

### **1.6.3.3 Lectins**

Studies have shown that the extracts of *H. hemerocallidea* Fisch. & C.A. Mey contains lectin-like compounds (Gaidamashvili and van Staden, 2002) and cytokinins (Hutchings *et al.*, 1996). Lectins are non-enzymatic glycoproteins that reversibly and specifically bind carbohydrate moieties on cell membranes (Gurib-Fakim, 2006). Lectins also called agglutinins react with surfaced-exposed carbohydrates of microbes and cause lectin-bacteria agglutination reactions to occur (Gaidamashvili and van Staden, 2002).

Due to their high specificity, lectins are used as diagnostic probes for the identification of bacterial pathogens as well as for the recognition and differentiation of malignant tumours. Studies on lectins have shown that lectins are capable of inhibiting the motility, multiplication and growth of some plant bacterial pathogens by agglutinins; thus lectins are perceived as important for plant defence system activation (Gaidamashvili and van Staden, 2002; Ghazarian *et al.*, 2011).

In humans, lectins prevent the adhesion and interaction of bacteria with the epithelial cells of the gastrointestinal tract. Lectin agglutinins can interact with enterocytes and lymphocytes in order to expose pathogens to body tissues, thus allowing for the activation of an immune response. Lectins are also believed to possess some anticancer properties together with apoptosis-inducing capabilities in cancer cells (Ghazarian *et al.*, 2011).

## 1.6.4 Pharmacological activities of *Hypoxis hemerocallidea* extracts

### 1.6.4.1 Anti-inflammatory and anti-diabetic activity

*H. hemerocallidea* Fisch. & C.A. Mey is a well-known remedy for the management and control of painful arthritic and inflammatory conditions and also for adult-onset type-2 diabetes. Supporting these claims, Ojewole (2006) showed that at a dose of 50–800 mg/kg body weight, oral administrations of *H. hemerocallidea* methanol and aqueous corm extracts significantly inhibited egg albumin-induced acute inflammation and also reduce blood glucose levels in both normal and streptozotocin-induced diabetic rats in a dose-dependent manner.

Furthermore, intraperitoneal injections of *H. hemerocallidea* extracts produced anti-nociceptive effects against chemically and thermally-induced nociceptive pain in mice (Ojewole, 2006). Researchers (Jäger *et al.*, 1996; Steenkamp *et al.*, 2006; Ncube *et al.*, 2012) demonstrated some inhibitory activity against COX-1 and COX-2 enzymes by both the ethanol and aqueous extracts of *H. hemerocallidea* in both *in vitro* and *in vivo* models. Neither of the same extracts produced a significant inhibition of the two enzymes *in vitro* even at concentrations as high as 0.5 mg/ml (Jäger *et al.*, 1996; Steenkamp *et al.*, 2006; Ncube *et al.*, 2012). Contrary to these findings, and according to Laporta *et al.*, (2007), rooperol showed a noticeable inhibitory effect on both enzymes, with a stronger effect on COX-2 than COX-1, yielding a COX-2/COX-1 IC<sub>50</sub> ratio of 1.9. Lectin-like proteins purified from aqueous extracts of *H. hemerocallidea* inhibit COX-1 enzyme that mediates prostaglandin synthesis *in vitro* (Gaidamashvili and van Staden, 2006). The anti inflammatory activity of *H. hemerocallidea* extracts are suggested to be more likely taking place via the inhibition of the prostaglandins, 5-lipoxygenase pathway and other inflammatory mediators such as antioxidant activity which in turn, inhibits COX enzymes (van der Merwe *et al.*, 1993; Steenkamp *et al.*, 2006).

The hypoglycaemic effects of *H. hemerocallidea* aqueous extracts were investigated on fasted normal and diabetic rats, with a dose-dependent (100–800 mg/kg) moderate to high response being recorded against insulin (5 mg/kg) and glibenclamide (5 mg/kg) as positive controls (Mahomed and Ojewole, 2003; Ojewole, 2006). At a dose of 800 mg/kg, the caused 30.2% and 48.5% reductions in the blood glucose concentrations of fasted normal and streptozotocin-treated diabetic rats respectively (Mahomed and Ojewole, 2003). They concluded that the extracts could likely induce hypoglycaemia by stimulating insulin release thereby enhancing the cellular uptake and utilization of glucose within the animal.

#### 1.6.4.2 Antioxidant activity

The aqueous and ethanolic extracts of *H. hemerocallidea* demonstrated good antioxidant properties through hydroxyl scavenging ability (Steenkamp *et al.*, 2006; Nair and Kanfer, 2007). *In vitro* studies indicate that aqueous extracts of *H. hemerocallidea* have good ability to scavenge free radicals (hydroxyl ions) (Mahomed and Ojewole, 2003). Rooperol significantly increased reactive oxygen species (ROS) and nitric oxide (NO) production, and phagocytosis in undifferentiated and differentiated human promonocytic U937 leukaemia cells than its positive control ascorbic acid (Boukes and van de Venter, 2012). Using the ferric reducing ability of plasma (FRAP) assay, the authors later confirmed the antioxidant capacity of rooperol, results of which were consistent with previous studies of Mahomed and Ojewole (2003), Steenkamp *et al.*, (2006) and Laporta *et al.*, (2007).

In a separate but related study, rooperol reduced quinolinic acid induced lipid peroxidation in rat liver homogenates and significantly scavenged the superoxide anion at pharmacological doses (Nair *et al.*, 2007). At similar concentrations (8, 16 and 32 mg/ml), rooperol demonstrated significantly greater ferric reducing activity than ascorbic acid (Nair *et al.*, 2007) while Boukes and van de Venter, (2012) reported similar results at a concentration of 20 mg/ml. Using albino rats, water decoction of *H. hemerocallidea* corms (10 mg/kg) exhibited good antioxidant activity by inducing protection against oxidative stress generated by chloroquine in a dose dependant manner (Chaturvedi and Mwape, 2011).

The bioactive compound rooperol isolated from *H. hemerocallidea* has a unique structural similarity to nordihydroguaiaretic acid which is a strong antioxidant, a phenomenon that could explain the antioxidant properties of these extracts (van der Merwe *et al.*, 1993; Boukes and van de Venter, 2012). Using platelet microsomes, rooperol and nordihydroguaiaretic acid gave comparable results in the inhibition of leukotriene synthesis in the polymorphonuclear leucocyte and prostaglandin synthesis as well as stimulate the oxidation of haemoglobin to a greater extent (van der Merwe *et al.*, 1993; Coetzee *et al.*, 1996).

#### 1.6.4.3 Antimicrobial and antiviral activity

A number of extracts from *Hypoxis* species have demonstrated antibacterial, antifungal and antiviral activities against numerous pathogenic strains associated with acute infections (Buwa and van Staden, 2006; Steenkamp *et al.*, 2006). Evidence-based laboratory investigations indicate that aqueous and alcoholic extracts of *H. hemerocallidea* possess a number of promising

pharmacological properties (Owira and Ojewole, 2009). Crude ethyl acetate extracts of *H. hemerocallidea* corms exhibited potent antimicrobial activities against *Staphylococcus aureus* and *Enterococcus faecalis* using a microdilution assay (Katerere and Eloff, 2008).

Following a similar test procedure, Ncube *et al.*, (2011) reported water and lipophilic leaf and corm extracts of *H. hemerocallidea* to have good antibacterial and anticandidal activity in autumn and winter against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* and *Candida albicans*. A remarkable complete inhibition of *Escherichia coli* growth at a concentration of 100 mg/ml was recorded from aqueous and methanolic extracts of *H. hemerocallidea* (Steenkamp *et al.*, 2006). Excellent agglutination activity against *Staphylococcus aureus* and *Bacillus subtilis* was reported from lectin-like proteins extracted from *H. hemerocallidea* (Gaidamashvili and van Staden, 2002).

In a synthetic membrane model study, the compound promoted high membrane leakage in *Escherichia coli* but with a much stronger inhibition on *Staphylococcus aureus*. The active compound rooperol exhibited a high physiological membrane leakage in an *Escherichia coli* model containing synthetic membranes. Inhibition of bacterial growth is through disruption of the phospholipid/ water interface through the formation of gel-fluid like intermediate structures with isotropic motion in phosphatidylglycerol membranes at physiological pH (Laporta *et al.*, 2007). *H. hemerocallidea* showed interference with the efflux of nevirapine across intestinal epithelial cells and potentially increase the bioavailability of this antiretroviral drug when taken concomitantly (Brown *et al.*, 2008).

#### **1.6.4.4 Cardiovascular and anticonvulsant activity**

In a study involving Chacma baboons, Coetzee *et al.*, (1996) established that a purified extract of *Hypoxis hemerocallidea* corms (rooperol) increased myocardial contractility *in vivo*. Rooperol caused moderate, transient increased cardiac output, stroke volume and vascular pressures without increased heart rate or filling pressures, suggestive of increased myocardial contractility probably allied to its catechol structure (Coetzee *et al.*, 1996).

Moreover, in their investigation on the effect of the aqueous corm extract of *H. hemerocallidea* on myocardial contractile performance of guinea-pig isolated atrial muscle strips *in vitro*, Ojewole *et al.*, (2006) reported significant concentration dependent positive inotropic and chronotropic responses. Furthermore, the extracts led to dose-related transient but significant ( $P < 0.05$ – $0.001$ )

reductions in the systemic arterial blood pressure and heart rates of hypertensive rats used in the same study (Ojewole *et al.*, 2006).

In another study, chronic ingestion of aqueous extract of *H. hemerocallidea* (as tea) was reported to have caused ventricular tachycardia in a 25-year-old male subject (Ker, 2005). The observations suggest that *H. hemerocallidea* corm extracts may contain some active chemical constituents with some cardio-stimulatory properties. Aqueous extracts of *H. hemerocallidea* (50–800 mg/kg) significantly delayed ( $P < 0.05$ – $0.001$ ) the onset of, an antagonized pentylenetetrazole-induced seizures in mice better than phenobarbitone and diazepam (which are known anticonvulsant compounds) (Ojewole, 2008). Stafford *et al.*, (2007) reported ethyl acetate extracts of *H. hemerocallidea* to exhibit moderate non-selective inhibitory effects at  $IC_{50}$  of  $25 \pm 5 \mu\text{g/ml}$  on monoamine-oxidase enzyme which is involved in a number of neurological processes.

#### **1.6.4.5 Anticancer activity**

Steenkamp and Gouws (2006) found that aqueous extracts of *H. hemerocallidea* at 50 mg/ml significantly inhibited DU-145 prostate cancer cell growth more than cisplatin, a known anti-tumor agent. Albrecht *et al.*, (1995) demonstrated that sitosterols from *H. hemerocallidea* have good *in vitro* activities by stimulating lymphocyte proliferative responses towards phyto-haemagglutinin at very low concentrations, making these compounds essential nutrients to be taken on a daily basis for the optimal functioning of the immune system.

*In vitro* studies on the compound hypoxoside have been shown to be cytotoxic against cancer cells at concentrations of up to 100 mg/ml. However, when hydrolyzed to its aglucone, rooperol, by  $\beta$ -3glucosidase, cytotoxicity was found to be ranging from concentrations between 2 and 10 mg/ml (Marini-Bettolo *et al.*, 1982). This led to the discovery of the promising potential of *H. hemerocallidea* as an oral prodrug for cancer therapy in humans given its first-pass metabolism into non-toxic conjugate rooperol which may be activated in tumor cells with high deconjugase activity (Albrecht *et al.*, 1995).

Evidence of rooperol's activity as an anticancer agent has been further demonstrated using a model system consisting of normal, non-invasive breast epithelial cell lines by spontaneous immortalization of cells from fibrocystic breast tissue support (Soule *et al.*, 1990; Tait *et al.*, 1990), and a premalignant, invasive and tumorigenic derivative, resulting in transfection of the cell line with a mutationally activated oncogene (Basolo *et al.*, 1991; Ochieng *et al.*, 1991).

In a study by Boukes and van de Venter (2011) to investigate and compare the cytotoxicity and mechanisms of action of *H. hemerocallidea* extract, the authors concluded that different cell lines exhibit different sensitivities towards the plant extract. They determined cytotoxicity of the *H. hemerocallidea* extract against cervical (HeLa), colorectal (HT-29) and breast (MCF-7) cancer cell lines as well as peripheral blood mononuclear cells (PBMCs). They also determined DNA cell cycle arrest in the Gap 1 synthesis or Gap 2 mitotic phase using propidium iodide staining. They concluded that the cytotoxic mechanism of *H. hemerocallidea* is exerted through the induction of cell cycle arrest and apoptosis (Boukes and van de Venter, 2011).

### **1.7 Problem statement**

ART has significantly improved the prognosis of HIV and AIDS infection by reducing transmission, viral load and limiting opportunistic infections. This has led to improved wellbeing and longevity of people living with HIV/AIDS (PLWHAs). However, ART results in toxicities that complicate its management and increases the cost of overall health care. Adverse effects have been reported with most antiretroviral drugs and are among the most common reasons for switching therapy (O'Brien *et al.*, 2003).

For instance, nephrolithiasis was seen in up to 27% of patients treated with indinavir and there are numerous studies demonstrating that tenofovir is also associated with impaired kidney function (Mocroft *et al.*, 2003). Glomerular filtration rate (GFR) correlates with the severity of kidney disease and typically decreases before the onset of symptoms of kidney failure (Mocroft *et al.*, 2003).

The HAART side effects have become an important public health problem contributing to more than 50% of kidney injuries, a fraction of which may require immediate transplantation (Choi *et al.*, 2009). It is anticipated that as the population of HIV-infected patients start to age and therefore remain on HAART for longer period of time, HIV and HAART-related metabolic disorders would correspondingly increase. The consequences are quite huge in terms of economic cost and care.

Jevtovic (2008) observed that the cumulative long-term toxicities of HAART emerge as a significant complication to the kidney. Finding possible mitigating avenues/ treatment options continue to attract scientific enquiring.

## 1.8 Justification for study

The problem of drug toxicity continues to attract the attention of researchers both in experiments and clinical trials. Evidence shows that animal models of drug toxicity represent a similar pattern in humans. For example, one-third of all drugs associated with hepatotoxicity in animals resulted in liver enzyme elevation in humans Sulkowski *et al.*, (2004).

Although, animal models may not always predict human drug toxicities, drug-induced toxicity described in clinical trials is often detected long after a drug may have entered the market (Sulkowski *et al.*, 2004). Therefore, experimental animal studies serves to investigate whether a drug has the potential for toxicity before even being tested in clinical trials. In this study, the rat serves as a suitable model for investigating the mammalian kidney based on its similar structure and physiologic functions, while being easy to handle and generally considered less expensive.

Treatment with cocktail of antiretroviral drugs predisposes People living with HIV and AIDS (PLWHAs) to adverse effects. Such cohorts of patients are at high risk of developing short and long-term complications such as renal-toxicity, hepatotoxicity, and cardiovascular disorders amongst other things (Sanders *et al.*, 2007).

The associated toxicities of HAART necessitate the need for safe and less costly adjuvants that can mitigate these effects. Plant-based extracts have the potential to fill this need. The search for medicinal plants has continued to grow due to its easy accessibility, relatively low cost and a perceived effectiveness. An increasing reliance on the use of medicinal plants in industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants (Fasinu *et al.*, 2012). The Southern African ecosystem is replete with medicinal plants that continue to drive improvement in indigenous knowledge systems.

It is estimated that about 80% of the general population in sub-Saharan Africa rely on African traditional medicines to treat various diseases (Hostetmann *et al.*, 2000). *H. hemerocallidea* Fisch. & C.A. Mey falls into this category and has been widely used for the treatment of various ailments within the region and studies have attributed this to the pharmacological properties it possesses (Albrecht *et al.*, 1995).

More so, there has been a surge in commercially available herbal remedies obtained as ‘over-the-counter’ medicines containing sterols with *H. hemerocallidea* extract enrichments claimed to be

efficacious against a variety of diseases and as an immune boosters for PLWHAs (Albrecht *et al.*, 1995). However, the scientific validation of these claims remains to be verified.

## **1.9 Research questions**

- i. Does *H. hemerocallidea* ameliorate kidney injuries following HAART?
- ii. Would vitamins C and E serve as a better antioxidant supplements than *H. hemerocallidea* in HAART-induced renal injuries?
- iii. What is/ are the probable mechanism(s) of HAART induced kidney injuries?

## **1.10 Objectives of study**

The objectives of this study are presented in line with two major focus areas:

- a. To assess the histological changes following administration of HAART on the kidney of experimental animals and the role of *H. hemerocallidea*.
- b. To obtain measures of renal function through assay of serum creatinine and blood urea nitrogen levels of the experimental animals following HAART and *H. hemerocallidea* treatment.

**CHAPTER TWO**  
**MANUSCRIPT FROM RESEARCH**

***Hypoxis hemerocallidea* does not attenuate renal injuries following highly active antiretroviral therapy**

Offor U, Jegede A.I, Onanuga I.O, Naidu E.C.S, Azu O.O

Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, South Africa.

Correspondence: azu@ukzn.ac.za

**Abstract**

Nephrotoxicity has become an important public health problem following highly active antiretroviral therapy (HAART), and there is paucity of literature reporting the attenuating influence of plant based adjuvant that can mitigate the effects. The study investigates the role *Hypoxis hemerocallidea* extract following HAART in an experimental animal model.

Sixty three adult male Sprague-Dawley rats were used for the study and were divided into 9 groups (A-I), where group A-H served as the experimental groups while group I served as the control. The experiment lasted for 56 days after which, the animals were sacrificed, the kidneys were harvested and prepared for H&E histological examination and blood samples were collected through cardiac puncture and centrifuge to get the serum for blood urea nitrogen and Serum creatinine analysis.

Organ-body weight ratios were significantly higher in group A and group F ( $p<0.05$ ).

Serum Creatinine (SCR) and blood urea nitrogen (BUN) levels were statistically elevated in HAART-treated animals ( $p<0.05$ ,  $0.001$ ). SCR levels in group D was significantly reduced ( $p<0.05$ ) but however, significantly elevated in groups B, C, G and H ( $p<0.001$ ). Groups B and C, as well as groups F and H resulted in higher BUN values ( $p<0.05$ ). The histological appearance of group A was highly compromised. When treated concomitantly with *Hypoxis hemerocallidea* (at both dosages), no attenuating influence was seen. However, low dose of *Hypoxis hemerocallidea* showed improved histological layout as compared to the high dose. Co-administration of HAART and combined dose of vitamin C and E did not improve the histoarchitecture.

Adjuvant treatment with *Hypoxis hemerocallidea* extract did not attenuate the nephrotoxicity of HAART in this model.

## Introduction

*Hypoxis hemerocallidea* (*H. hemerocallidea*) Fisch. & C.A. Mey belongs to the family Hypoxidaceae, is widely distributed in the savanna regions of South Africa, Swaziland and Zimbabwe and is increasingly also grown as a pot plant. It was previously known as *Hypoxis rooperi* and commonly called the African potato, Lilabatseka, Zifozonke (Swazi) and Inkomfe (Zulu) (Drews *et al.*, 2008; van Wyk *et al.*, 2008).

The *H. hemerocallidea* is a stemless, geophytic, perennial herb with large corms (tubers) which are dark brown to black on the outside and bright yellow inside (van Wyk *et al.*, 2008) with more than 90 species endemic to South Africa (Fabian and Germishuizen, 1997). The corm of *H. hemerocallidea* has been successfully used to treat some traditional western ailments including urinary tract infections, hypertension, a variety of inflammatory conditions, testicular tumors, and more recently as immune boosters for HIV-AIDS. Its traditional usage dates back many generations (Owira and Ojewole, 2009). There is also anecdotal evidence that the plant can be poisonous (Ker, 2005).

The corm of *H. hemerocallidea* has been exploited in a variety of ways; dietary/herbal, traditional and pharmaceutical purposes (Awad and Fink, 2000). The Hypoxideae family especially the *H. hemerocallidea* is noted for the occurrence of a hypoxide which is a secondary metabolite of the plant (Nair *et al.*, 2007; Drews *et al.*, 2008) that is hydrolysed into rooperol (which is the active and powerful antioxidant component of the corm) (Laporta *et al.*, 2007). Rooperol is also been reported to be anticarcinogenic against the mouse model BL6 melanoma (Albrecht *et al.*, 1995).

Extracts of *H. hemerocallidea* corms have been ingested by man as a dietary supplement hence used for its nutritional value. With increasing interest in the use of phytosterols for the reduction of serum cholesterol and for immune boosting, there has been a resultant increase in scientific investigations (Moreau *et al.*, 2002) surrounding these benefits. More so, there has been a surge in commercially available herbal remedies obtained as ‘over-the-counter’ medicines containing sterols with *H. hemerocallidea* extract enrichments claimed to be efficacious against a variety of diseases.

However, the scientific validation of these claims remains to be verified despite its anti-inflammatory, antimicrobial, anticonvulsant and anticancer properties that various authors (Ojewole *et al.*, 2006; Steenkamp *et al.*, 2006; Ncube *et al.*, 2011) report but none on any antiretroviral protocol.

The significant achievements in reduction in morbidity and mortality following the intervention with highly active antiretroviral therapy (HAART) on HIV/AIDS epidemic has led to a dramatic step that improved longevity of people living with HIV/AIDS (PLWHAs). Despite these positive achievements, associated HAART-induced organ toxicities continue to accumulate thus dampening the perceived impacts (Bodeker *et al.*, 2006).

The most common and troublesome toxicities of HAART includes hepatotoxicity (Abrescia *et al.*, 2005; Soriano *et al.*, 2008) linked to mitochondrial damage especially in patients treated with zidovudine, stavudine or didanosine (Walker *et al.*, 2004), anaemia and metabolic disorders especially with protease inhibitors (Sattler *et al.*, 2001), nephrotoxicity especially with nevirapine or tenofovir (Wyatt *et al.*, 2009). HAART-induced kidney injuries could manifest in electrolyte and acid-base imbalance as well as lactic acidosis and occur via mechanisms that target direct tubular toxicity, allergic reactions and precipitation of insoluble drug crystals within renal tubular lumens (Parazella *et al.*, 2010).

Like most toxic drugs, oxidative stress mediates most pathophysiological processes especially with the use of antiretroviral drugs. The reactive oxygen species produced directly act on cell components including lipids, proteins and DNA and destroy their structure. The consequence of this is oxidative stress and cell death (Azu, 2012).

We hypothesis that the oxidative stress-induced HAART renal injuries can be mitigated by adjuvant treatment with extracts of *H. hemerocallidea* due to the rich antioxidative properties. Therefore, our study will investigate the role of adjuvant use of AP in HAART protocol on renal architecture in view of the fact that there is paucity of data supporting this interaction despite its use by PLWHAS.

## **Materials and methods**

### **Chemicals/drugs**

Lamivudine (3TC), stavudine and nevirapine (Aspen) and vitamin C (L-ascorbic acid) were bought from Pharmicare Ltd, Port Elizabeth, South Africa and of analytical grade. Vitamin E solution was obtained from Kyron Prescription CC, Benrose, Johannesburg, South Africa.

### **Plant**

Fresh corms of *H. hemerocallidea* were purchased from a local 'Muthi' in Umbilo Road, Durban, KwaZulu-Natal, harvested in July, 2014. The corms were taken to the herbarium unit of the Department of Life Science, Westville Campus, University of KwaZulu-Natal, Durban South Africa and were authenticated by Prof. Ashley Nicholas. Sample of it was retained at the herbarium for future reference.

### **Preparation of corm aqueous extract**

*H. hemerocallidea* fresh corms were extracted according to the procedure of Ojewole *et al.*, 2009. They were washed with water, cut into smaller pieces, air-dried at room temperature (25-28°C) and ground into fine particles in a commercial blender.

The grinded corm was soaked in hot distilled water and extracted twice, on each occasion with 2.5 litres of hot distilled water (at 90–100 °C) for 12 hours. The combined extract, were concentrated to dryness under reduced pressure in a rotary evaporator at  $70 \pm 1$  °C. The resulting crude aqueous extract was freeze-dried, finally giving off a dark brown, and powdery aqueous extract residue (170.5 g, 85.25 % yield). Without any further purification, aliquot portions of the aqueous extract residue were weighed and dissolved in distilled water (at room temperature) for use on each day of our experiments.

### **Animal treatment and experimental design**

Sixty- three adult male Sprague-Dawley rats weighing between 261-310 g were used for this study. The animals were bred and maintained at the Animal House of the Biomedical Resources Unit, University of KwaZulu-Natal, South Africa. The animals received humane care in accordance with the Principle of Laboratory Animal Care of the National Medical Research Council and the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institute of Health Guide, 1985). The experimental procedures was approved by the University of

KwaZulu-Natal Animal Ethics Committee and assigned reference number: 100/14/Animals. The investigation was carried out at the Biomedical Research Unit Animal housing facility.

All the rats were housed in well ventilated plastic cages (7 rats per cage) having dimensions of (52 cm long × 36 cm wide and 24 cm high). They were maintained under standardized animal house conditions (temperature: 28–31 °C; light: approximately 12 h natural light per day) and were fed with standard rat pellets from (Meadow feeds a Division of Astral Operations Limited, Durban, South Africa) and given tap water *ad libitum*. The animals were randomly distributed to nine treatment groups: A, B, C, D, E, F, G, H, I with seven animals per group.

Group A: HAART (a cocktail of Lamivudine, Stavudine and Nevirapine) using the human therapeutic dose equivalents (mg/day for Lamivudine, Stavudine and Nevirapine respectively) administered as a daily dose (Azu *et al.*, 2014).

Group B received HAART and AP extract (100 mg/kgbw),

Group C received HAART and AP extract (200 mg/kgbw),

Group D received HAART and vitamin C (250 mg/kgbw),

Group E received HAART and vitamin E (40 mg/kgbw; Bansal *et al.*, 2005), twice a week for the 8 weeks,

Group F received combination of HAART, vitamin C and vitamin E,

Group G received AP extract alone (100 mg/kgbw),

Group H received AP extract alone (200 mg/kgbw),

Group I served as the control administered 0.9 % normal saline.

All administration was done daily by oro-gastric gavage except for vitamin E which was administered intramuscularly. At the end of the treatment period (56 days), the animals were killed 24 hr after the last treatment under excess Halothane<sup>®</sup> anaesthesia.

### **Body and kidney weight**

Body weights of animals were recorded on the first day before treatment (initial), thereafter weekly and on the day of sacrifice (final). Kidney weight (KW) was measured by an electronic balance (Mettler Toledo; Microsep (Pty) Ltd., Greifensee, Switzerland).

### **Histological examination of tissues**

Twenty four hours after the last treatment, the animals were given humane sacrifice under excess Halotane<sup>®</sup> anaesthesia and the kidneys were removed and weighed. They were examined for gross

pathology and immediately fixed in 10% Neutral buffered saline. After proper fixation, the tissues were dehydrated in graded series of alcohol, cleared in Xylene and embedded in paraffin wax using a cassette. The tissues were sectioned at 4 µm thickness using Leica RM 2255 microtome and stained with haematoxylin and eosin. The stained slides were cover slipped using DPX mounting glue directly over the tissue section ensuring no air bubbles were trapped. Thereafter, the slides were left overnight to dry for examination under light microscope. The sections were examined using a binocular Olympus microscope coupled with digital image camera Nikon Eclipse 50i, Tokyo, Japan (used to acquire the images). An independent histopathologist blinded to the treatment groups reported on the qualitative assessment of the slides.

### **Assessment of kidney function**

Blood samples were collected through cardiac puncture and allowed to clot for 30 minutes and centrifuged for 15 minutes at 3000 revolutions per minute. The serum was decanted into Eppendorf tubes and prepared for analysis of blood urea nitrogen (BUN) and serum creatinine (SCR) using Beckman Coulter Synchron® system(s) BUN and SCR assay kit.

Beckman Coulter Synchron® system BUN assay kit and Beckman Coulter Synchron® system SCR assay kit were bought from Global Viral Laboratory, Durban, South Africa for the assessment of BUN and SCR levels.

### **Statistical analysis**

Continuous variables (kidney and body weight, BUN and SCR levels), were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post-test using Graph pad prism ® statistical software 5.02. The results are expressed as mean ± SD (standard deviation). Values were considered significant at  $p < 0.05$ .

### **Results**

**Mortality:** No animal died during the experimental period.

### **Body weight and organ (kidney) weight changes**

The final body weights of rats were in all groups higher than the initial weight but the percentage gain was maximal in group D (HAART with vitamin C), then groups H, G all recording 48.66 %, 46.38 % and 44.08 % respectively. Least weight gain was observed in group B animals treated with

HAART +AP 100mg/kg (table 1). Similarly, kidney weight changes were not significant except for group A that recorded a significant increase ( $p<0.05$ ) and group B that recorded lowest body weight when compared with the control. Organ-body weight ratios were significantly higher in HAART treated animals and HAART with vitamins C and E ( $p<0.05$ ) compared to other groups. Whereas group E (HAART and vitamin E) and group G (*H. hemerocallidea* 100 mg/kg) did not show any increase in organ-body weight ratios as compared to control (table 1). Group H animals treated with *H. hemerocallidea* 200 mg/kg showed significant reduction ( $p<0.05$ ) in organ-body weight ratios. Co-administration of *H. hemerocallidea* (at both dosages) with HAART resulted in increased organ-body ratio, however not significant.

Table 1: Body weight, kidney weight and KW/BW ratio of experimental and control groups

Group	Initial BW (g)	Final BW (g)	BW difference (g)	BW difference (%)	KW (g)	KWBR
A	311.43 ± 3.36	427.00 ± 9.94	115.57	37.11	2.48 ± 0.19	0.58*
B	279.57 ± 7.16	363.14 ± 11.50	83.57	29.89	2.06 ± 0.26	0.57
C	281.57 ± 2.88	382.71 ± 5.52	101.14	35.92	2.17 ± 0.12	0.57
D	261.00 ± 7.49	388.00 ± 8.28	127.00	48.66	2.17 ± 0.14	0.56
E	271.57 ± 7.14	359.71 ± 12.31	88.14	32.46	1.93 ± 0.24	0.54
F	273.00 ± 6.29	371.86 ± 18.43	98.86	36.21	2.16 ± 0.33	0.58*
G	256.71 ± 8.65	369.86 ± 12.66	113.15	44.08	1.99 ± 0.26	0.54
H	260.00 ± 6.06	381.00 ± 11.21	121.00	46.38	2.03 ± 0.15	0.53*
I	285.86 ± 3.80	407.29 ± 3.69	121.43	42.48	2.18 ± 0.45	0.54

Values are expressed as mean ± SD for each group and considered statistically significant at  $p<0.05$ \*. BW= body weight of rats, KW= kidney weight of rats, KWBR= kidney weight body ratio.

### SCR and BUN and levels

Functional nephrotoxicity indices such as blood urea nitrogen (BUN) and serum creatinine (SCR) were statistically elevated in HAART-treated animals ( $p<0.05$ , 0.001) compared with the control. Adjuvant treatment with *H. hemerocallidea* (at both dosages) with HAART, as well as co-treatment with vitamins C and E and HAART as well as *H. hemerocallidea* 200mg/kg resulted in higher BUN values ( $p<0.05$ ) (table 2). SCR levels of vitamin C + HAART group was significantly reduced

( $p < 0.05$ ) when compared with the control. Similarly, SCR values were significantly elevated in groups B, C, G and H ( $p < 0.001$ ) (table 2).

Table 2: Effects of adjuvants of *H. hemerocallidea*, vitamins C and E with HAART on creatinine and urea levels in the rats

Groups	BUN (mmol/l)	CR-S ( $\mu\text{mol/l}$ )
A	8.07 $\pm$ 0.98*	38.67 $\pm$ 2.31 <sup>+</sup>
B	7.13 $\pm$ 1.62*	41.33 $\pm$ 1.15 <sup>+</sup>
C	6.77 $\pm$ 1.95*	38.00 $\pm$ 2.00 <sup>+</sup>
D	4.66 $\pm$ 0.42	23.67 $\pm$ 2.08 <sup>+</sup>
E	5.27 $\pm$ 0.65*	32.33 $\pm$ 3.05*
F	5.86 $\pm$ 0.93	30.00 $\pm$ 3.00
G	5.10 $\pm$ 1.92*	34.33 $\pm$ 1.52 <sup>+</sup>
H	6.63 $\pm$ 1.03*	34.33 $\pm$ 4.04 <sup>+</sup>
I	4.90 $\pm$ 0.26	30.67 $\pm$ 1.52

Values are expressed as means  $\pm$ SD of each group. \*  $p < 0.05$ ; <sup>+</sup> $p < 0.001$ ; BUN is blood urea nitrogen; SCR is serum creatinine.

### Histopathology of the Kidney

Histopathological sections of kidneys in control, HAART with vitamin E, and low dose of *H. hemerocallidea* revealed adequate preservation of tubular structures with normal glomeruli and Bowman's capsule. The interstitial spaces and the urinary space were of normal architecture (figure 5, 7 and 9). Animals treated with high dose of *H. hemerocallidea* showed variable glomerular and tubular degenerations varying from; glomerular basement thickening, interstitial inflammation, tubular cell swelling and hypercellularity (figure 8). Histopathological sections of kidney treated with HAART cocktail also showed cytoarchitectural patterns with extensive necrosis of glomerular apparatus with degeneration in the tubules (figure 1). Equally, sections of kidney tissue treated with adjuvant *H. hemerocallidea* (both doses) showed similar patterns with the HAART group, generalized necrosis of renal glomerular corpuscles with many indistinguishable tubules in the section (figure 2 and 3). Animals treated with vitamin C as adjuvants also showed some degenerate

/atrophic renal tubules (figure 4). Combination of vitamins C and E with HAART (figure 6) resulted in many glomeruli congestion with mild to moderate necrosis and interstitial infiltrations.

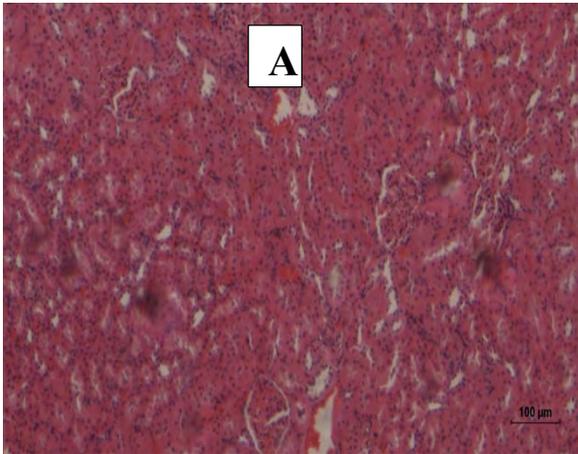


Fig 1: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with HAART cocktail. H and E stains. Note the necrosis in glomerulus, atrophied proximal convoluted tubules, distal convoluted tubules and Bowman's capsular atrophy.

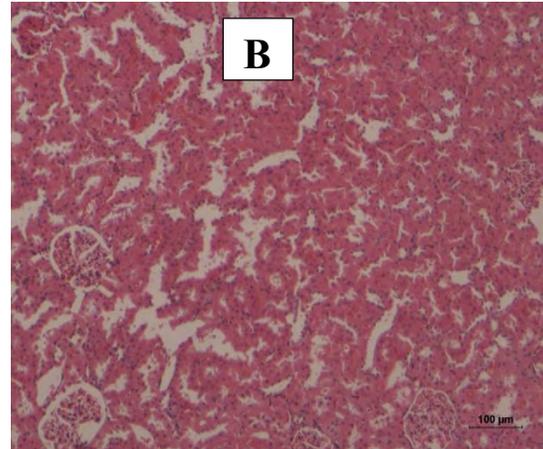


Fig 2: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with HAART and low dose of *H. hemerocallidea* extract. H and E stains. Note: Cellular distortion of glomeruli with atrophy and disorganization of proximal convoluted tubules and distal convoluted tubules.

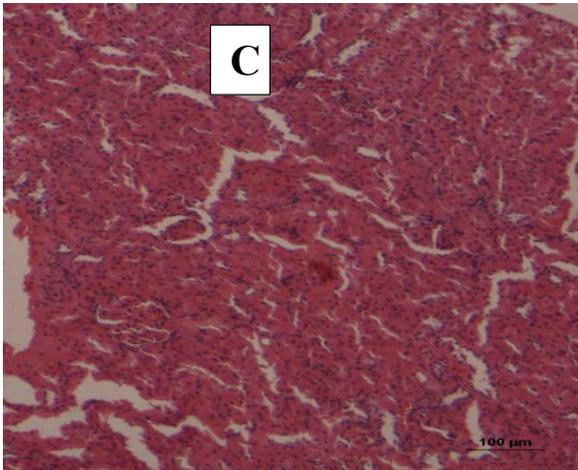


Fig 3: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with HAART and high dose of *H. hemerocallidea* extract. H and E stains. Note: Disorganization in proximal & distal cellular architecture with generalized hyperplasia.

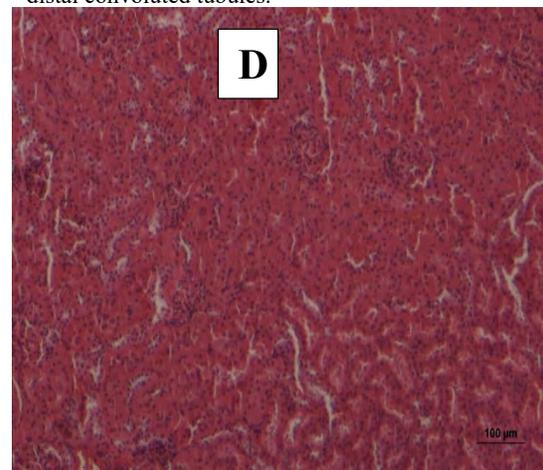


Fig 4: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with HAART and vitamin C. H and E stains. Note: prominent necrosis in the glomerulus with deranged Bowman's capsule and disorganization in the proximal & distal convoluted tubules.

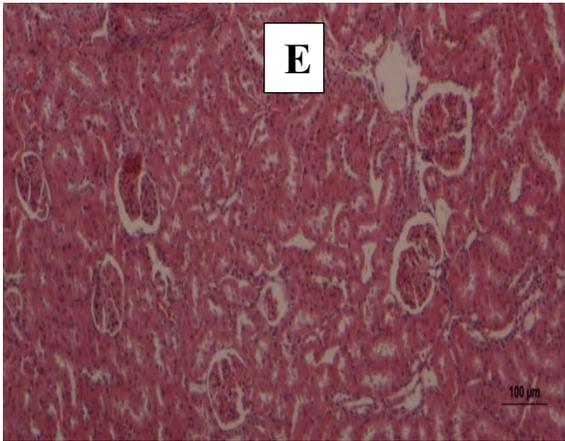


Fig 5: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with HAART and vitamin E. H and E stains. Histoarchitecture essentially normal.

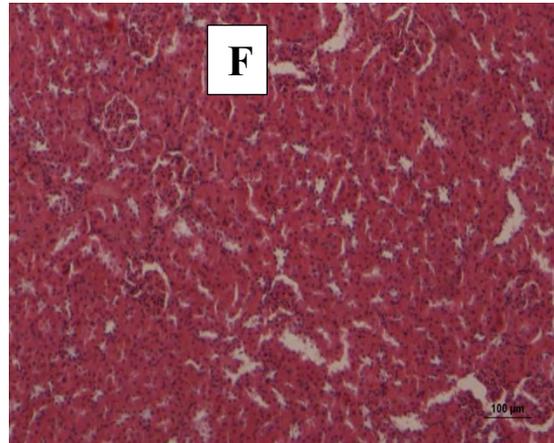


Fig 6: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with HAART and vitamin (C and E). H and E stains. Note: Cellular distortion of proximal and distal convoluted tubules with generalized hypercellularity.

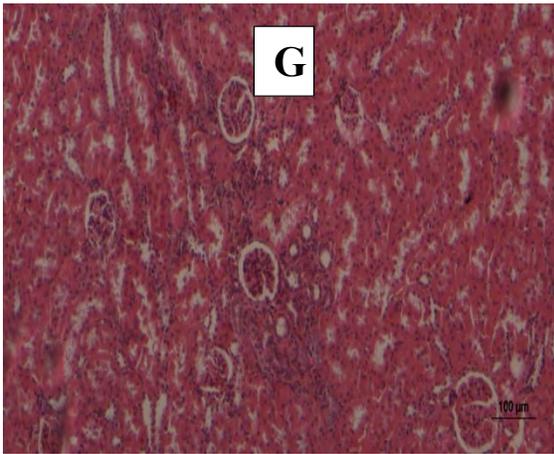


Fig 7: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with low dose of *H. hemerocallidea* extract alone. H and E stains. Histoarchitecture is normal.

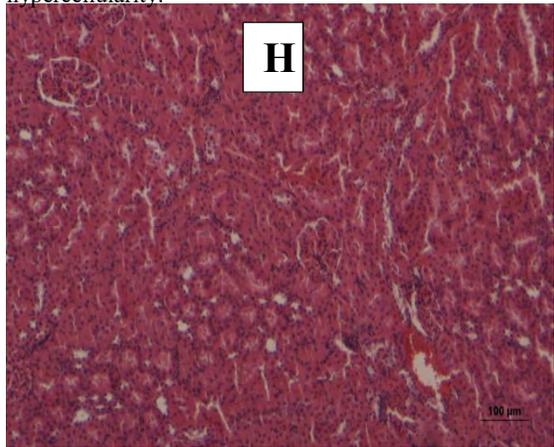


Fig 8: Photomicrograph of cross section of kidney of Sprague-Dawley rats treated with high dose of *H. hemerocallidea* extract alone. H and E stains. Note: Cellular distortion of glomeruli with derangement of Bowman's capsule and space and atrophy of the proximal and distal convoluted tubules.

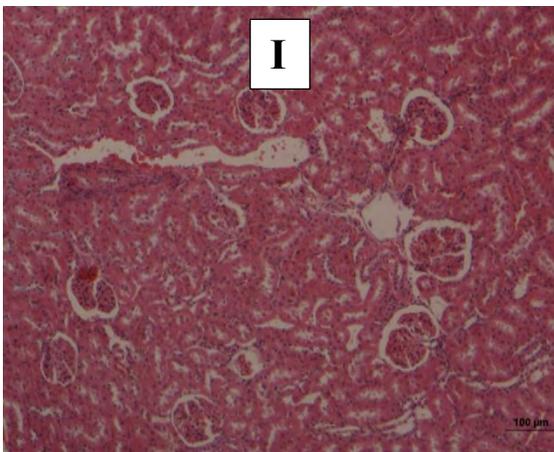


Fig 9: Photomicrograph of cross section of kidney of Sprague-Dawley rats in control group. Showing normal histoarchitecture, H and E stains.

## Discussion

The kidney plays an important role in the metabolism and excretion of drugs as well as maintenance of constancy in the volume and composition of the extracellular fluid (ECF). Through these mechanisms, the basic functions of glomerular filtration, tubular reabsorption and secretions are fulfilled (Skalova, 2005) but also predisposes this organ to various types of injuries that may require renal replacement therapy (Choi *et al.*, 2009). The animal model of assessment of renal function especially using the male rats has proven to be suitable due to the less variability in intra-renal enzyme distribution (Raab, 1972).

As PLWHAs continue to enjoy decreased morbidity and mortality due to HAART, organ toxicities (in particular the kidney) are frequently becoming a major concern in view of associated consequences that may predispose to hypertensive and /or other metabolic complications (Azu, 2014). The result of this study showed that animals on HAART as well as adjuvant treatment with *H. hemerocallidea* recorded increased body weight. Similarly, kidney weight was increased in HAART treated animals. The reason for this weight gain remains to be explained but it is possible that appetite was not affected in the animals by the treatment. However the significant kidney-body weight ratio possibly suggests of negative effects on the kidney. Nephrotoxicity may manifest in the form of organ swelling and hence increase kidney weight as seen in the kidney-body weight ratio (table 1) which is a sensitive and effective indicator of toxicology and thus important in the identification of target organ by toxicants (Asagba *et al.*, 2007). While daily urinary flow was not monitored, it is plausible to adduce that polyuria with consequent loss of water and ions might be (in part) responsible for the weight changes of which *H. hemerocallidea* could not ameliorate.

Morphological and physiological studies have identified the renal system as site of attack by noxious substances/drugs with consequent derangement on the renal histology. More so, nephrotoxicity has been identified as a potential flaw with some antiretroviral regimen (especially with nevirapine or tenofovir) (Wyatt *et al.*, 2009). Therefore, the aberrations in the glomerular histo-architecture in HAART-treated group as well as the inability of adjuvant *H. hemerocallidea* to mitigate the derangements further suggest that the later may not be nephroprotective. This observation is supported by the reports of Musabayane *et al.*, (2005) that showed that chronic infusion of *H. hemerocallidea* extracts may cause a decrease in glomerular filtration rate and elevate plasma creatinine concentrations in rats, suggesting an impairment of kidney functions.

However, treatment with low dose *H. hemerocallidea* alone did not show any damage to the renal histoarchitecture and other parameters recorded whereas treatment with high dose *H. hemerocallidea* showed aberrant histoarchitecture of the kidney with extensive derangements of the glomerulus and the renal tubular system.

Interestingly, result on co-administration of vitamins C and E with HAART does not support (from histopathological observations and biochemical markers) the concurrent use of these vitamins as supplements by PLWHAs. It is believed that vitamins C and E alter bioavailability, metabolism and pharmacokinetics of certain HIV medications raising concerns about the possibility of HAART and vitamin supplementation leading to increased toxicity (Nkengfack *et al.*, 2012). While this report does not include oxidative stress parameters, there is substantial evidence from *in vitro* animal studies as well as clinical trials recognizing oxidative stress as a major pathway of action for most antiretroviral drugs. The consequence is direct cellular attack and mitochondrial DNA depletion or direct tubular cytotoxicity (Gallant and Deresinski 2003; Cihlar *et al.*, 2001).

Biochemical markers play an important role in accurate diagnosis of diseases and also for assessing risks and adopting therapies that may improve clinical outcome. Over decades research and utilization of biomarkers have evolved substantially. As markers of renal function, serum creatinine (SCR) and blood urea nitrogen (BUN) routinely serve as indicators for normal biological, pathologic processes, or pharmacologic responses to a therapeutic intervention. The present study revealed that administration of HAART caused marked impairment in renal function. SCR and BUN concentrations were significantly higher in HAART-treated rats and this is consistent with lower creatinine and BUN clearance. In addition, elevated level of SCR and BUN concentrations might have resulted from remarkable leakage due to hypercellularity of the glomeruli and tubules. It is speculated that the capacity for glomerular filtration and tubular absorption may have been altered, thus bringing about functional overload of nephrons with subsequent renal dysfunction.

Interestingly, the administration of HAART and *H. hemerocallidea* concomitantly did not improve the SCR and BUN clearance in this protocol. It is possible that drug-drug interactions between *H. hemerocallidea* and HAART that could affect efficacy or safety of the later is possible. Mills and his team (2005) showed that interactions between extracts of *H. hemerocallidea* and antiretroviral drugs inhibit CYP3A4, an isoform of cytochrome P450 and drug transporter protein (P-glycoprotein). In addition, *H. hemerocallidea* extracts have been claimed to activate drug nuclear receptor pregnane X (PXR) which modulates expressions of both CYP3A4 and p-glycoprotein

(Mills *et al.*, 2005). Many antiretroviral drugs are substrate of CYP3A4 and some herbal preparations are known to alter blood levels of these drugs through their effects on CYP3A4 and p-glycoprotein (Mills *et al.*, 2005). It is therefore reasonable to argue that *H. hemerocallidea* could potentially interact with HIV drug- metabolizing enzymes thus patients taking *H. hemerocallidea* extracts concurrently with antiretroviral drugs, may be at risk of developing adverse effects which may lead to treatment failure, viral resistance and/ or drug toxicity.

## **Conclusion**

While HAART continues to be the cornerstone in the management of HIV/AIDS in sub-Saharan Africa, the complex issues of toxicities, resistance as well as drug interactions would continue to be an impediment to achieving the desirable goals. It has emerged from this study that renal derangements based on the histological and biochemical assessments were seen following HAART and these were not mitigated by *H. hemerocallidea* adjuvants and therefore draws to caution the concomitant use of *H. hemerocallidea* extracts by PLWHAs in the sub-region. However, because *H. hemerocallidea* may contain several other constituents which are yet to be isolated apart from rooperol, hypoxoxide, lectins and phytosterols, there is need for further investigation in this direction.

## **Acknowledgement**

We thank the College of Health Sciences, University of KwaZulu-Natal Durban South Africa for the postgraduate financial support awarded to the second author and the National Research Foundation of South Africa for the grant, Unique No. 94018 awarded to the senior author.

## **References**

- Abrescia N, D'Abbraccio M, Figoni M, Busto A, Maddaloni A and De Marco M, (2005). Hepatotoxicity of antiretroviral drugs. *Current Pharmaceutical Design. Review*. 11(28): 3697-3710.
- Albrecht C.F, Theron E.J and Kruger P.B, (1995). Morphological characterization of the cell growth inhibitory activity of rooperol and pharmacokinetic aspects of hypoxoside as an oral prodrug for cancer therapy. *S. Afri Med Journal* 85:853-860.
- Asagba S.O, Adaikpoh M.A, Kadiri H and Obi FO, (2007). Influence of aqueous extract of *Hibiscus sabdariffa* L. petal on cadmium toxicity in rats. *Biol Trace Elem Res* 115: 47-57.

Awad A.B and Fink C.S, (2000). Phytosterols as anticancer dietary components: evidence and mechanism of action. *J. Nutri* 130: 2127-2130.

Azu O.O, (2012). Highly Active Antiretroviral Therapy (HAART) and testicular morphology: current status and a case for stereological approach. *J. Androl* 33: 1130-1142.

Azu O.O, (2012). Highly Active Antiretroviral Therapy (HAART) and testicular morphology: current status and a case for stereological approach. *J. Androl* 33: 1130-1142.

Bodeker G, Carter G, Burford G and Dvorak-Little M, (2006). HIV/AIDS: Traditional Systems of Health Care in the Management of a Global Epidemic. *J. Altern Complement Med.* 12(6): 63-76.

Choi A.I, Shlipak M.G, Hunt P.W, Martin J.N and Deeks S.G, (2009). “HIV-infected persons continue to lose kidney function despite successful antiretroviral therapy,” *AIDS*, 23(16): 2143–2149.

Cihlar T, Ho E.S, Lin D.C and Mulato A.S, (2001). Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. *Nucleosides Nucleotides Nucleic Acids*, 20: 641-648.

Drewes S.E, Elliot E, Khan F, Dhlamini J.T.B and Gcumisa M.S.S (2008). *Hypoxis hemerocallidea* not merely a cure for benign prostate hyperplasia. *J. Ethnopharmacol* 119: 593-598.

Fabian A and Germishuizen G, (1997). *Wild flowers of Northern South Africa*. Fernwood press Vlaeberg. 285.

Gallant J.E and Deresinski S, (2003). “Tenofovir disoproxil fumarate,” *Clinical Infectious Dis.* 37(7): 944– 950.

Ker J, (2005). Ventricular tachycardia as an adverse effect of the African potato (*Hypoxis* Spp). *Cardiovasc J.S. Afr* 16: 55.

Laporta O, Perez-Fons L, Mallavia R, Caturla N and Micol V, (2007). Isolation, characterization and antioxidant capacity assessment of the bioactive compounds derived from *Hypoxis rooperi* corm extract (African potato). *Food Chem* 101: 1425-1437.

Mills E, Cooper C, Seely D and Kanfer I, (2005). African herbal medicines in the treatment of HIV: Hypoxis and sunderlandia. An overview of evidence and pharmacology. Nutrition Journal 4:19.

Moreau R.A., Bruce D.W. and Kevin B.H. (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog. Lipid Res. 41, 457–500.

Musabayane C.T, Xozwa K and Ojewole J.A.O, (2005). Effects of *Hypoxis hemerocallidea* (Fisch & C.A. Mey) (Hypoxidaceae) corm (African potato) aqueous extract on renal electrolyte and fluid handling in the rat. Renal failure 2: 763-770.

Nair V.D.P and Kanfer I, (2007). A capillary zone electrophoresis method for the quantitative determination of hypoxoside in commercial formulations of African potato (*Hypoxis hemerocallidea*). Phytochem. Anal. 18: 475-483.

National institutes of Health Guide for the care and use of Laboratory Animals. DHEW Publication (NIH) (1985): Revised, Office of Science and Health Reports, DRR/NIH, Bethesda; USA.

Ncube B, Finnie J.F, and van Staden J, (2011). Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. S. Afr. J. Bot.77: 387-396.

Nkengfack G.N, Torimiro J.N, and Englert H, (2012). Effects of antioxidants on CD4 and viral load in HIV-infected women in sub-Saharan Africa—Dietary supplements vs. local diet. Int. J. Vitam. Nutr. Res. 82: 63–72.

Ojewole J.A.O, Olayiwola G and Nyinawumuntu A, (2009). Bronchorelaxant property of ‘African potato’ (*Hypoxis hemerocallidea* corm) aqueous extract in vitro. J. smooth muscles research 45: 241-248.

Ojewole J.A.O, Kamadyaapa D.R and Musabayane C.T, (2006). Some in vitro and in vivo cardiovascular effects of *Hypoxis hemerocallidea* Fisch & C.A. Mey (Hypoxidaceae) corm (African potato) aqueous extract in experimental animal models. Cardiovascular J. South Afri. 17: 166-171.

- Owira P.M.O and Ojewole A.O, (2009). 'African potato' (*Hypoxis hemerocallidea* corm): a plant medicine for modern and 21<sup>st</sup> century diseases of mankind? A review. *Phytotherapy Research* 23: 147-152.
- Perazella M.A, (2010). "Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy," *Kidney International*. 78(11):1060–1063.
- Raab W.P, (1972). Kaliopenic nephrosis: protective action of l-ornithine-l-aspartate. *Clinica Chimica Acta*. 39 (1): 239-242.
- Skalova S, (2005). The diagnostic role of urinary N-acetyl-b-glucosaminidase (NAG) activity in the detection of renal tubular impairment. *Acta Medica*.48:75-80.
- Soriano V, Puoti M, Garcia-Garsco P, Rockstroh J.K, Benhamou Y, Barreiro P and McGovern B, (2008). Antiretroviral drugs and liver injury. *AIDS*.22:1–13.
- Sattler F.R, Qian D, Louie S, Johnson D, Briggs W, DeQuattro V and Dube M.P, (2001). Elevated blood pressure in subjects with lipodystrophy. *AIDS* 15, 2001–2010.
- Steenkamp V, Gouws M.C, Gulumian M, Elgorashi E.E and van Staden J, (2006). Studies on antibacterial, anti-inflammatory and antioxidant activity of herbal remedies used in the treatment of benign prostrate hyperplasia and prostatitis. *J. Ethnopharmacol*. 103: 71-75.
- Van Wyk B.E (2008). A broad review of commercially important South African medicinal plants. *J. Ethnopharmacology* 119: 342-355.
- Walker U, Bauerle J, Laguno M, Murillas J, Mauss S, Schmutz G, Setzer B, Miquel R, Gatell J.M and Mallolas J, (2004). Deletion of mitochondrial DNA in liver under antiretroviral therapy with didanosine, stavudine or zalcitabine. *Hepatology*. 39:311–317.
- Wyatt C. M., Morgello S and Katz-Malamed R, (2009). The spectrum of kidney disease in patients with AIDS in the era of antiretroviral therapy. *Kidney Int'l*. 75(4):428–434.

## CHAPTER THREE

### SYNTHESIS, CONCLUSION AND RECOMMENDATION

#### 3.1 Synthesis

There is no doubt that HAART has been the most important progress in the management of HIV-infected patients in the last decade. A number of observations suggest that the beneficial effects of HAART include lowering the viral load of HIV-infected patients by generally targeting reverse transcription of viral RNA into cDNA and hence, their positive impact on longevity. However, the growing population of patients treated with HAART requires the consideration of potential renal side effects that may accompany the use of HAART.

Histological studies are important in renal localization of the toxic or beneficial actions of various drugs. In this study, the histological parameters of the HAART cocktail present a compromised histological state with extensive necrosis of the glomeruli, tubular toxicity as well as atrophy of the Bowman's capsule and space. The observation of the toxicity of HAART are in tandem with reports by other authors who reported deleterious histological changes following HAART on the testicular tissue linked to the damage of the mitochondrial apparatus (Azu *et al.*, 2014).

Therefore, it is tempting to speculate that the toxicity of HAART in the renal localization is due to mitochondrial cytopathy. Several studies of HAART renal toxicity argue in favour of such hypothesis (Kakuda *et al.*, 2000).

Firstly, among recognized adverse effects of HAART, mitochondrial toxicity as well as damage indicated by histological features in the kidney is induced by members of NRTIs family (Kakuda *et al.*, 2000). To a greater or lesser degree, all are responsible for proximal tubular cell toxicity that clinically manifest as proximal tubular dysfunction. Therefore it is tempting to make a link between the tubular cell toxicity and mitochondrial toxicity of HAART.

Secondly, HAART-treated patients often present with renal failure clinically and histologically related to tubulointerstitial nephropathy and associated with extra-renal manifestations attributable to mitochondrial cytopathy (Coca and Perazella, 2002).

Thirdly, intracellular droplets-like lipids are present in proximal tubular cells of patients with renal toxicity of tenofovir or adefovir (Creput *et al.*, 2003). It is well established that intra-cytoplasmic

lipid droplets are characteristic of unused fatty acids in cells with defective mitochondria (Kakuda *et al.*, 2000).

Finally, the “mitochondrial cytopathy hypothesis” was invoked to explain adefovir and stavudine nephrotoxicity by Tanji *et al.*, 2001 who reported a patient with degenerative changes in proximal tubules and renal tubular cells with dysmorphic mitochondria and mitochondria DNA depletion.

Adjuvant treatment with *H. hemerocallidea* and HAART in both doses showed deleterious histological changes of the kidney. This possibly could suggest drug-drug interactions. Drug and traditional medicine (TM) interactions occur when a TM and orthodox medicine influence the effects of each other. This may cause a change in pharmacodynamics and / or pharmacokinetic parameters which may affect efficacy or safety of either orthodox medicine or TM. (Mills *et al.*, 2005). Potential drug interactions between extracts of *H. hemerocallidea* and antiretroviral drugs have been reported to inhibit CYP3A4 isoform of cytochrome P<sub>450</sub> and drug transporter protein (P-glycoprotein). Patients taking *H. hemerocallidea* concurrently with antiretroviral drugs, may therefore, be at risk of developing adverse effects which may lead to treatment failure.

Treatment with high dose of *H. hemerocallidea* showed aberrant histoarchitecture of the kidney with extensive derangements of the glomerulus and the renal tubular system whereas treatment with low dose of *H. hemerocallidea* did not show any damage to the renal histoarchitecture whereupon, the remarkable renal indices were intact.

The use of vitamins C and E as antioxidant supplements in ART has been practiced both in experimental and clinical conditions because they are believed to scavenge free radicals. However, vitamins C and E (combined) did not show any improvement of the kidney from the various parameters assessed. Treatment with vitamin E alone shows essentially normal histological appearance while vitamin C did not show any improvement in renal histoarchitecture further raising concerns about the possibility of HAART and vitamin supplementation leading to increased toxicity.

Although this report does not include oxidative stress parameters, evidence from literatures recognizes oxidative stress as a major pathway of action for most antiretroviral drugs. Oxidative stress can also promote the formation of a variety of vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient; and thus reducing glomerular filtration rate (GFR) (Garcia Cohen *et al.*, 2000). A low GFR leading to a condition of reduced urinary excretion, results in retention and

accumulation of various nitrogenous waste products in the plasma and other body fluids (Garcia Cohen *et al.*, 2000). The present study revealed that administration of HAART caused marked impairment in renal function. SCR and BUN concentrations were significantly higher in HAART treated rats and this is consistent with lower creatinine and BUN clearance.

Weight coefficient (organ/BW ratio) is a sensitive and effective indicator of toxicology and thus important in the identification of target organ by toxicants (Asagba *et al.*, 2007). The study revealed that the weight coefficient of kidney tissue in HAART- treated animals was significantly higher than those of controls, further supporting the toxicity of HAART to this organ.

### **3.2 Conclusion**

HAART has dramatically improved survival in PLWHAs. However, precautions should be taken to prevent HAART-related nephrotoxicity or systemic life-threatening side-effects whose risk may be increased by renal failure. The study highlighted that treatment with *H. hemerocallidea* in preventing renal toxicity following HAART led to more deleterious changes in the histoarchitecture of the kidney. From the study, *H. hemerocallidea* and HAART may not be taken together in the face of kidney life- threatening situations following HAART, and thereby putting the patients on the horns of more dilemmas. From available folkloric, anecdotal and laboratory evidence, *H. hemerocallidea* extracts contain some chemical compounds with anti-inflammatory, antidiabetic and antioxidant activities. They may also contain several other constituents which are yet to be isolated. It is possible that these chemical compounds of *H. hemerocallidea* extract act together with other compounds yet to be isolated. Certainly, more laboratory and clinical studies are required to clarify this situation.

### **3.3 Recommendations**

In view of the findings of this research on nephrotoxicity of HAART regimen, it is believed that the occurrence of renal derangements are prevalent, HIV patients who are clinically and virologically stable should continue with HAART unless severe or complex complications emerge. If they occur, adequate treatment options for the adverse events are prescribed like regimen change, temporary withdrawal or complete stoppage after a thorough clinical evaluation.

Secondly, in view of the possible drug-drug interactions likely between AP and HAART regimen, it is recommended that further studies be carried out on the specific active component of AP on this

protocol and detailed pharmacokinetic properties thereof. This in addition to other investigational parameters would shed light on the possible mechanisms of action of AP in this model.

## REFERENCES

- Abegaz B.M, Ngadjui B.T, Bezabih M, Mdee L.K (1999).** Novel natural products from marketed plants of eastern and Southern Africa. *Pure and applied chemistry* 71: 919-926
- Abrescia N., D'Abbraccio M., Figoni M., Busto A., Maddaloni A., De Marco M, (2005).** Hepatotoxicity of antiretroviral drugs. *Current Pharmaceutical Design. Review.* 11(28): 3697-3710.
- Ahmad M, (2006).** “Abacavir-induced reversible Fanconi syndrome with nephrogenic diabetes insipidus in a patient with acquired immunodeficiency syndrome”. *J. Postgrad Med.* 52(4): 296–297.
- Albrecht C.F, Theron E.J, Kruger P.B (1995).** Morphological characterization of the cell growth inhibitory activity of rooperol and pharmacokinetic aspects of hypoxoside as an oral prodrug for cancer therapy. *S. Afri Med Journal* 85:853-860.
- Al-kahtani, M.A, Zuleta C, Caviedes-Vidal E, Garland T, Jr. (2004).** "Kidney mass and relative medullary thickness of rodents in relation to habitat, body size, and phylogeny". *Physiol and Biochem Zoology* 77 (3): 346–365.
- Aruoma O.I, Sun B, Fuji H, Neergheen V.S, Bahorun T, Kang K.S, Sung M.K, (2006).** Low molecular proanthocyanidin dietary biofactor oligonol: Its modulation of oxidative stress, bioefficacy, neuroprotection, food application and chemoprevention potentials. *Biofactors* 27, 245-265.
- Asagba S.O, Adaikpoh M.A, Kadiri H and Obi FO, (2007).** Influence of aqueous extract of *Hibiscus sabdariffa* L. petal on cadmium toxicity in rats. *Biol Trace Elem Res* 115: 47-57.
- Ashford S.L, (2006).** How HIV and AIDS affect populations. Population reference Bureau. Website: [www.prb.org](http://www.prb.org). cited on 14th January, 2013.
- Awad A.B, Fink C.S, (2000).** Phytosterols as anticancer dietary components: evidence and mechanism of action. *J. Nutri* 130: 2127-2130.

**Azu O.O, Naidu E.C.S, Naidu J.S, et al., (2014).** Testicular histomorphologic and stereological alterations following short-term treatment with highly active antiretroviral drugs (HAART) in an experimental animal model. *J. Androl.* 2(5):772-779.

**Azu O.O, (2012).** Highly Active Antiretroviral Therapy (HAART) and testicular morphology: current status and a case for stereological approach. *J. Androl* 33: 1130-1142.

**Azu O.O, Duru F.I.O, Osinubi A.A, Oremosu A.A, Norohna C.C, Okanlawon A.O and Elesha S.O, (2010).** Histomorphometric effects of *Kigelia Africana* (Bignoniaceae) fruit extract on the testis following short-term treatment with cisplatin in Sprague-Dawley rats. *Middle East Fertil Soc J.* 15: 200-208.

**Bachmann S, Sakai T, Kriz W (1998).** Nephron and collecting duct structure in the kidney of rat. Springer Berlin Heidelberg. Pp 3-24. ISBN-978-3-642-96956.

**Bansal AK, Bansal M, Soni G & Bhatnagar D, (2005).** Protective role of vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. *Chem-Biol Interact* 156, 101–111.

**Basolo F, Elliot J, Tait L, Chen Q.C, Maloney T.M, Russo L.H, Pauley R, Momiki S, Caamano J, Klein-Szanto A.J.P, Koszalka M, Russo J, (1991).** Transformation of human breast epithelial cells by c-Ha-ras oncogene. *Mol. Carcinogenesis* 4, 25-35.

**Bentley M.L, Corwin H.L, Dasta J, (2010).** Drug-induced acute kidney injury in the critically ill adult: recognition and prevention strategies. *Crit Care Med* 38(6):169-174.

**Bodeker G, Carter G, Burford G, Dvorak-Little M, (2006).** HIV/AIDS: Traditional Systems of Health Care in the Management of a Global Epidemic. *J. Altern Complement Med.* 12(6): 63-76.

**Bonnet F, Balestre E, Bernardin E, Pellegrin J.L, Neau D, Dabis F, (2005).** “Risk factors for hyperlactataemia in HIV infected patients, Aquitaine Cohort, 1999–2003,” *Antiviral Chemistry and Chemotherapy.* 16(1): 63–67.

**Bouic P.J.D, Etsebeth S, Liebenberg R.W, Albrecht C.F, Pege K, van Jaarsveld P.P, (1996).** Beta-sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte

proliferation: implications for their use as an immunomodulatory vitamin combination. *Int'l. J. immunopharmacol* 18: 693-700.

**Boukes G.J, van der Venter M, Oosthuizen V, (2008).** Quantitative and qualitative analysis of sterols/sterolins and hypoxoside contents of three Hypoxis (African potato) Spp. *Afri. J. Biotech.* 7: 1624-1629.

**Boukes G.J, van de Venter M, (2011).** Cytotoxicity and mechanisms of action of Hypoxis Spp. (African potato) against HeLa, HT-29 and MCF-7 cancer cell lines. *J. Med Plants* 5: 2766-2774.

**Boukes G.J, van de Venter M, (2002).** Rooperol as an antioxidant and its role in the innate immune system: an in vitro study. *J. Ethnopharmacol.* 144: 692-699.

**Brown L, Heyneke O, Brown D, van Wyk J.P.H, Hamman J.H (2008).** Impact of traditional medicinal plant extracts on antiretroviral drug absorption. *J. Ethnopharmacol.* 199, 588-592.

**Bruce M. Carlson (2004).** *Human Embryology and Developmental Biology* (3<sup>rd</sup> Ed.). Saint Louis: Mosby. ISBN 0-323-03649.

**Bunnell R, Cherutich P, (2008).** Universal HIV testing and counseling in Africa. *Lancet.* 28: 2148-2150.

**Buwa L.V, van Staden J, (2006).** Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *J. Ethnopharmacol.* 103: 139-142.

**Campbell L.J, Ibrahim F, Fisher M, Holt S. G, Hendry B. M, Post F. A, (2009).** "Spectrum of chronic kidney disease in HIV-infected patients," *HIV Medicine.* 10(6): 329–336.

**Carcelain G, Li T, Autran B, (1999).** Immune reconstitution under highly active antiretroviral therapy (HAART). *AIDS Review.* 1:51-56.

**CDC, (2009).** 2007 Disease Profile. National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention. Atlanta, GA. 7-14.

**Chaturvedi P, Mwape M.P (2011).** Effect of African potato (*Hypoxis hemerocallidea*) extract on oxidative stress induced by chloroquine in albino rats. *Afr. J. Food Agric. Nutr. Dev.* 11, 4476-4489.

**Choi A.I, Shlipak M.G, Hunt P.W, Martin J.N, Deeks S.G, (2009).** “HIV-infected persons continue to lose kidney function despite successful antiretroviral therapy,” *AIDS*, 23(16): 2143–2149.

**Chugh S, Bird R, Alexander E.A, Leonard J.M, Heath-Chiozzi M, (1997).** “Ritonavir and renal failure,” *The New Engl J. Med.* 336(2): 138.

**Cihlar T, Ho E.S, Lin D.C, Mulato A.S, (2001).** Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. *Nucleosides Nucleotides Nucleic Acids*, 20: 641–648.

**Clapp, W.L, Zhou X.J, Laszik Z, Nadasdy T, D'Agati V.D (2009).** *Renal Anatomy.* Silva's Diagnostic Renal Pathology NY: Cambridge University Press. 44:72-76.

**Cocas and Perazella M.A, (2002).** Rapid communication: Acute renal failure associated with tenofovir: Evidence of drug-induced nephrotoxicity. *Am J Med Sci* 324:342–344.

**Coetzee J.F, Krugger P.B, Albrecht C.F, Vahed N, van Jaarsveld P.P, (1996).** Pharmacokinetic behavior and cardiovascular effects of intravenously administered hypoxoside and rooperol. *Arzneimittelforschung.* 46: 997-1000.

**Cooper R.D, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M, (2010).** Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis.* 51(5): 496–505.

**Couzigou C, Daudon M, Meynard J.L, (2007).** “Urolithiasis in HIV-positive patients treated with atazanavir,” *Clin Infect Dis.* 45(8):105-108.

**Creput C, Gonzalez-Canali G, Hill G, et al., (2003).** Renal lesions in HIV-1-positive patient treated with tenofovir. *Aids* 17:935–937.

**Crum-Cianflone N, Ganesan A, Teneza-Mora N, (2010).** Prevalence and factors associated with renal dysfunction among HIV-infected patients. *AIDS Patient Care and STDs*. 24: 353–360.

**d’Arminio M.A., Lepri, A.C., Rezza, G., Pezzotti P., Antinori A., Phillips, A., Angarano G., Colangeli V., De Luca A., Ippolito G., Caggese L., Soscia F., Filice G., Gritti F., Narciso P., Tirelli U, Moroni M, (2000).** Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients. I.CO.N.A. Study Group. *Italian Cohort of Antiretroviral-Naïve Patients. AIDS*, 14: 499–507.

**Daugas E, Rougier J.P, Hill G, (2005).** HAART-related nephropathies in HIV-infected patients. *Kidney International*, 67: 393–403.

**De Visser R, Grierson J, (2002).** Use of alternative therapies by people living with HIV/AIDS in Australia. *AIDS Care*. 14(5): 599-606.

**Dori L, Buonomini A. R, Viscione M, Sarmati L, Andreoni M, (2010).** “A case of rhabdomyolysis associated with raltegravir use”. *AIDS*. 24(3): 473–475.

**Drewes S.E, Elliot E, Khan F, Dhlamini J.T.B, Gcumisa M.S.S (2008).** *Hypoxis hemerocallidea* not merely a cure for benign prostate hyperplasia. *J. Ethnopharmacol* 119: 593-598.

**Emery S, Neuhaus J.A, Phillips A.N, Babiker A, Cohen C.J, Gatell J.M, (2008).** Major clinical outcomes in antiretroviral therapy (ART)-naïve participants and in those not receiving ART at baseline in the SMART study. *J. Infect Dis*. 197: 1133-1144.

**Fasinu P.S, Bouic P.J, Rosenkranz B, (2012).** An overview of the evidence and mechanisms of herb–drug interactions. *Front Pharmacol*. 63:71.

**Friedman and Young, (2000).** *Effects of Disease on Clinical Laboratory Tests*, 5th ed. IFCC.

**Gaidamashvili M, van Staden J, (2006).** Prostaglandin inhibitory activity by lectin like proteins from South African medicinal plants. *S. Afr. J. Bot*. 72: 661-663.

**Gaidamashvili M, van Staden J, (2002).** Interaction of lectin-like proteins of South African medicinal plants with *Staphylococcus aureus* and *Bacillus subtilis*. *J. Ethnopharmacol*. 80:131-135.

**Gallant J.E, Deresinski S, (2003).** “Tenofovir disoproxil fumarate,” *Clinical Infectious Dis.* 37(7): 944– 950.

**Garcia-Cohen E. C, Marin J, Diez-Picazo L. D, Baena A. B, Salaices M and Rodriguez-Martinez M. A, (2000).** Oxidative stress induced by tertbutylhydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: role of cyclooxygenase2 isoform J *Pharmacol Exp Ther.* 293(1): 75-81.

**Ghazarian H, Idoni B, Oppenheimer S.B, (2011).** A glycobiology review: Carbohydrates, Lectins and implications in cancer therapeutics. *Acta histochemica* 113: 236-247.

**Glodny B, Unterholzner V, Taferner B *et al.*, (2009).** "Normal kidney size and its influencing factors - a 64-slice MDCT study of 1.040 asymptomatic patients". *BMC Urology* 9 (1): 10-19.

**Gurib-Fakim A, (2006).** Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of medicine* 27: 1-9

**Gupta S.K, (2008).** “Tenofovir-associated Fanconi syndrome: review of the FDA adverse event reporting system,” *AIDS Patient Care and STDs.* 22(2): 99–103.

**Hawkins T, (2010).** Understanding and managing adverse effects of antiretroviral therapy. *Antiretroviral Research.* 85: 201-209.

**Herlitz L.C, Mohan S, Stokes M.B, Radhakrishnan J, D’Agati V.D, Markowitz G.S, (2010).** “Tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities,” *Kidney International.* 78(11): 1171–1177.

**Hostetmann K, Martson A, Ndjoko K, Wofender J.L, (2000).** The potential of African plants as a source of drugs. *Curr Org Chem* 4:973-1010.

**<https://aidsinfo.nih.gov/education.../fact.../fda-approved-hiv-medicines>** (Last updated 4/28/2015; last reviewed 4/28/2015).

**Hutchings A, Scott A.H, Lewis G, Cunningham A, (1996).** Zulu medicinal plants- an inventory. University of Natal Press, Pietermaritzburg, South Africa. 55-56.

**Jager A.K, Hutchings A, van Staden J, (1996).** Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. *J. Ethnopharmacol.* 52: 95-100.

**Jevtovic D. J, (2008).** The prevalence and risk of hepatitis flares in a Serbian cohort of HIV and HCV co-infected patients treated with HAART. *Biomed and Pharmacotherapy,* 62: 21-25.

**Joshi M. K, Liu H. H, (2000).** “Acute rhabdomyolysis and renal failure in HIV-infected patients: risk factors, presentation, and pathophysiology,” *AIDS Patient Care and STDs.* 14(10): 541–548.

**Kakuda T.N, (2000).** Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clin Ther* 22:685–708.

**Katerere D.R, Eloff J.N, (2008).** Anti-bacterial and antioxidant activity of *Hypoxis hemerocallidea* (Hypoxidaceae): can leaves be substituted for corms as a conservation strategy? *S. Afr. J. Bot.* 74: 613-616.

**Kayode A.A, Kayode O.T, Aroyeun O.A, Stephen M.C, (2011).** Hematological and hepatic enzyme alterations associated with acute administration of antiretroviral drugs. *J. Pharmacol Toxicol.* 6(3):293–302.

**Ker J, (2005).** Ventricular tachycardia as an adverse effect of the African potato (*Hypoxis* Spp). *Cardiovasc J.S. Afr* 16: 55.

**Krishnan M, Nair R, Haas M, Atta M.G, (2000).** “Acute renal failure in an HIV-positive 50-year-old man,” *American J. Kidney Dis.* 36(5): 1075–1078.

**Kushnir V.A, Lewis W, (2011).** Human immunodeficiency virus, acquired immunodeficiency syndrome and infertility: emerging problems in the era of highly active antiretrovirals. *Fertil Steril.* 96(3):546–550.

**Labarga P, Barreiro P, Martin-Carbonero L, et al., (2009).** “Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir”. *AIDS.* 23(6): 689–696.

**Laporta O, Perez-Fons L, Mallavia R, Caturla N, Micol V, (2007).** Isolation, characterization and antioxidant capacity assessment of the bioactive compounds derived from *Hypoxis rooperi* corm extract (African potato). *Food Chem* 101: 1425-1437.

**Mahomed I.M, Ojewole J.A.O, (2003).** Hypoglycaemic effect of *Hypoxis hemerocallidea* corm (African potato) aqueous extract in rats. *Methods and findings in experimental & clinical pharmacology* 25: 617-623.

**Mallal S, Phillips E, Carosietal G, (2008).** “HLA-B\*5701 screening for hypersensitivity to abacavir,” *The New Engl J. Med.* 358(6): 568–579.

**Marini-Bettolo G.B, Patamia M, Nicolette M, Galeffi C, Messina L, (1982).** Research on African medicinal plants: Hypoxoside, a new glycoside of unknown structure from *Hypoxis obtusa* Busch. *Tetrahedon* 38: 1683-1687. Mocrift A, Ledergerber B, Katlama C, Kirk O, Reiss P, d’Arminio M.A, Knysz B, Dietrich M, Phillips A.N, Lundgren J.D (2003). EuroSIDA study group. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. *Lancet.* 362:22–29.

**Mills E, Cooper C, Seely D, Kanfer I, (2005).** African herbal medicines in the treatment of HIV: *Hypoxis* and *sunderlandia*. An overview of evidence and pharmacology. *Nutrition Journal* 4:19

**Milkowski A, (2004).** Working concept on Reproductive Health/HIV and AIDS in Kenya.

**Montessori V, Press N, Harris M, Akagi L, Montaner J.S.G, (2004).** Adverse effects of antiretroviral therapy for HIV infection. *Canadian Med Assoc J.* 170: 2.

**Murphy M.D, Hearn M.O, Chou S, (2003).** “Fatal lactic acidosis and acute renal failure after addition of tenofovir to an antiretroviral regimen containing didanosine,” *Clin Infect Dis.* 36(8): 1082–1085.

**Musabayane C.T, Xozwa K, Ojewole J.A.O, (2005).** Effects of *Hypoxis hemerocallidea* (Fisch & C.A. Mey) (Hypoxidaceae) corm (African potato) aqueous extract on renal electrolyte and fluid handling in the rat. *Renal failure* 2: 763-770.

**Nair V.D.P, Kanfer I, (2007).** A capillary zone electrophoresis method for the quantitative determination of hypoxoside in commercial formulations of African potato (*Hypoxis hemerocallidea*). *Phytochem. Anal.* 18: 475-483.

**Nair V.D.P, Dairam A, Agbonon A, Arnason J.T, Foster B.C, Kanfer L, (2007).** Investigation of the antioxidant activity of African potato (*Hypoxis hemerocallidea*). *J. Agric. Food chem.* 55: 1707-1711.

**Nair V.D.P, Kanfer I, (2006).** High-performance liquid chromatographic method for the quantitative determination of hypoxoside in African potato (*Hypoxis hemerocallidea*) and in commercial products containing the plant material and/or its extracts. *J. Agric and Food Chem* 54: 2816-2821.

**NASCOP, (2002).** Clinical Guidelines on antiretroviral Therapy. NASCOP, Nairobi.

**National institutes of Health Guide for the care and use of Laboratory Animals. DHEW Publication (NIH) (1985):** Revised, Office of Science and Health Reports, DRR/NIH, Bethesda; USA.

**Ncube B, Finnie J.F, van Staden J, (2011).** Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. *S. Afr. J. Bot.*77: 387-396.

**Ncube B, Finnie J, van Staden J, (2002).** Seasonal variation in the cyclooxygenase inhibitory activities of four South African medicinal bulbs. *S. Afr. J. Bot.* 78: 246-251.

**Ndong T.A, Wadouachi A, Sangwan-Norreel B.S, Sangwan R.S, (2006).** Efficient in vitro regeneration of fertile plants from corm ex-plants of *Hypoxis hemerocallidea* (African potato). *Plant cell Reports* 25: 265-273.

**Nelson M, Azwa A, Sokwala A, Harania R.S, Stebbing J, (2008).** Fanconi syndrome and lactic acidosis associated with stavudine and lamivudine therapy. *AIDS.* 22: 1374–1376.

**Nkengfack G.N, Torimiro J.N, and Englert H, (2012).** Effects of antioxidants on CD4 and viral load in HIV-infected women in sub-Saharan Africa—Dietary supplements vs. local diet. *Int. J. Vitam. Nutr. Res.* 82: 63–72.

**O'Brien M.E., Clark R.A., Besch C.L., Myers L, Kissinger P, (2003).** Patterns and correlates of discontinuation of the initial HAART regimen in an urban out-patient cohort. *J.AIDS*, 34: 407–414.

**Ochieng J, Basolo F, Albini A, Melchiori A, Watanabe H, Elliot J, Raz A, Parodi S, Russco J, (1991).** Increased invasive, chemotactic and locomotive abilities of c-Ha-ras transferred human breast epithelial cells. *Invasion Metastasis* 11, 38-47.

**Ojewole J.A.O, Olayiwola G, Nyinawumuntu A, (2009).** Bronchorelaxant property of 'African potato' (*Hypoxis hemerocallidea* corm) aqueous extract in vitro. *J. smooth muscles research* 45: 241-248.

**Ojewole J.A.O, (2008).** Anticonvulsant activity of *Hypoxis hemerocallidea* Fisch. & C.A. Mey. (Hypoxidaceae) corm (African potato) aqueous extract in mice. *Phytother. Res.* 22: 91-96.

**Ojewole J.A.O, (2006).** Antinoceptive, anti-inflammatory and antidiabetic properties of *Hypoxis hemerocallidea* Fisch & C.A. Mey (Hypoxidaceae) corm (African potato) aqueous extract in mice and rats. *J. Ethnopharmacol.* 103: 126-134.

**Ojewole J.A.O, Kamadyaapa D.R, Musabayane C.T, (2006).** Some in vitro and in vivo cardiovascular effects of *Hypoxis hemerocallidea* Fisch & C.A. Mey (Hypoxidaceae) corm (African potato) aqueous extract in experimental animal models. *Cardiovascular J. South Afri.* 17: 166-171.

**Ojewole J.A.O, (2002).** Anti-inflammatory properties of *Hypoxis hemerocallidea* corm (Africa potato) extracts in rats. *Methods and findings in experimental & clinical pharmacology* 24: 685-687.

**Oti S.O, Mutua M, Mgomella G.S, Egondi T, Ezeh A, Kyobutungi C, (2013).** HIV mortality in urban slums of Nairobi, Kenya 2003–2010: a period effect analysis. *BMC Public Health.* 13-18.

**Overton E. T, Nurutdinova D, Freeman J, Seyfried W, Mondy K. E, (2009).** "Factors associated with renal dysfunction within an urban HIV-infected cohort in the era of highly active antiretroviral therapy". *HIV Medicine.* 10(6): 343–350.

**Owen-Smith A, Diclemente R, Wingood G, (2007).** Complementary and alternative medicine use decreases adherence to HAART in HIV-positive women. *AIDS Care.* 19(5): 89-93.

**Owira P.M.O, Ojewole J.A.O, (2009).** ‘African potato’ (*Hypoxis hemerocalidea* corm): a plant-medicine for modern and 21<sup>st</sup> century diseases of mankind? A review. *Phytother. Res.* 23: 147-152.

**Palella Jr F.J.J, (1998).** Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N. Engl. J. Med.*, 338: 853–860.

**Pegel K.H, (1997).** The importance of sitosterol and sitosterolin in human and animal nutrition. *S. Afri. J. Sci.* 93: 263-268.

**Peltzer K, Preez N.F.D, Ramlagan S, Fomundam H, Anderson J, Chanetsa L, (2011).** Antiretrovirals and the use of traditional, complementary and alternative medicine by HIV patients in Kwazulu-Natal, South Africa: A Longitudinal Study. *Afr J Tradit Complement Altern Med.* 8(4): 37-45.

**Perazella M.A, (2010).** “Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy,” *Kidney International.* 78(11):1060–1063.

**Perazella M.A, (2009).** Renal vulnerability to drug toxicity. *Clin J. Am Soc Nephrol.* 4(7):1275-1283.

**Pezzotti P, (1999).** Increasing survival time after AIDS in Italy: the role of new combination antiretroviral therapies. Tuscany AIDS Study Group. *AIDS,* 13: 249– 255.

**Raab W.P, (1972).** Kaliopenic nephrosis: protective action of l-ornithine-l-aspartate. *Clinica Chimica Acta.* 39 (1): 239-242.

**Reid A, Wolfgang A, Walker S, Ian G, Kityo W, Hughes C, Kambugu P, Gilks A, Mugenyi C. F, Munderi P, Hakim P. J, Diana M. G, (2008).** Severe Renal Dysfunction and Risk Factors Associated with Renal Impairment in HIV-Infected Adults in Africa Initiating Antiretroviral Therapy. *HIV/AIDS.* 46: 1271-1281.

**Rock R.C, Walker G.W, Jenning D.C, (1986).** Nitrogen metabolites and Renal function. *Fundamentals of Clinical Chemistry,* 3<sup>rd</sup> ed, Philadelphia, WB Saunders Company. 673-690.

**Roe J, Campbell L. J, Ibrahim F, Hendry B.M, Post F.A, (2008).** “HIV care and the incidence of acute renal failure,” *Clin Infect Dis.* 47(2): 242–249.

**Romer, Alfred Sherwood, Parsons, Thomas S, (2000).** *The Vertebrate Body.* Philadelphia, PA: Holt-Saunders International. Pg. 367–376. ISBN 0-03-910284.

**Saag M.S, Kilby J.M, (1999).** HIV-1 and HAART: a time to cure, a time to kill. *Nat Med.* 5:609–611.

**Sadler T.W, (2003).** *Langman’s medical embryology.* Lippincots Williams & Wilkins.9 ed. Pg 321-324. ISBN 0-7817-4310-9.

**Sanders E.J, Graham S.M, Okuku H.S, (2007).** HIV-1 infection in high risk men who have sex with men in Mombasa, Kenya. *AIDS,* 21: 2513–2520.

**Sattler F.R, Qian D, Louie S, Johnson D, Briggs W, DeQuattro V and Dube M.P, (2001).** Elevated blood pressure in subjects with lipodystrophy. *AIDS* 15, 2001–2010.

**Sembulingam K and Sembulingam P, (2006).** *Essentials of medical physiology.* Jaypee Brothers 4<sup>th</sup> ed. Pg 275. ISBN 81-8061-125-6.

**Shah M, (2004).** Use of complementary and alternative medicine by HIV/AIDS patients. *GUJHS.* 1(3).

**Shisana O, Rehle T, samba L.C, Zuma K, Jooste S, Zungu N, Labadarios D, Onoya D, (2014).** *South African National HIV Prevalence, Incidence and Behaviour Survey, 2012.* Cape Town, HRSC Press. Pp 32.

**Skalova S, (2005).** The diagnostic role of urinary N-acetyl-b-glucosaminidase (NAG) activity in the detection of renal tubular impairment. *Acta Medica.*48:75-80.

**Smith B.J, Albrecht C.F, Liebenberg R.W, Kruger, P.B, Freestone M, Gouws L, Theron E, Bouic P.J.D, Atsebeth S, van Jaarsveld P.P, (1995).** A phase 1 trial of hypoxoside as an oral prodrug for cancer therapy- absence of toxicity. *S. Afri. Med. J.* 85: 865-870.

**Soriano V, Puoti M, Garcia-Garsco P, Rockstroh J.K, Benhamou Y, Barreiro P, McGovern B, (2008).** Antiretroviral drugs and liver injury. *AIDS*.22:1–13.

**Soule H, Maloney T.M, Wolman S.R, Peterson Jr W.D, Berenz R, McGrath C.M, Russo J, Pauley R.J, Jones R.F, Brooks S.C, (1990).** Isolation and characterization of a spontaneously immortalized human breast epithelial cell line MCF-10. *Cancer Res.* 50: 6075-6086.

**Southern African Development Community (SADC), (2002).** SADC ministerial consultative meeting on nutrition and HIV/AIDS. SADC: Johannesburg.

**Stafford G.I, Pedersen P.D, Jager A.K, van Staden J, (2007).** Monoamine oxidase inhibition by Southern African traditional medicinal plants. *S. Afr. J. Bot.* 73: 384-390.

**Steenkamp V, Gouws M.C, (2006).** Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. *S. Afr. J. Bot.* 72: 630-633.

**Steenkamp V, Gouws M.C, Gulumian M, Elgorashi E.E, van Staden J, (2006).** Studies on antibacterial, anti-inflammatory and antioxidant activity of herbal remedies used in the treatment of benign prostrate hyperplasia and prostatitis. *J. Ethnopharmacol.* 103: 71-75.

**Suave N, Dzokoto A, Opare B, Kaitoo E.E, Khonde N, Mondor M, Bekoe V, and Pe´pin J, (2002).** The price of development: HIV infection in a semiurban community of Ghana. *J Acquir Immune Defic Syndr.* 29:402–408.

**Sulkowski M.S, (2004).** Drug-induced liver injury associated with antiretroviral therapy that includes HIV-1 protease inhibitors. *Clin Infect Dis* 38: 90– 97.

**Szczzech L.A, Gupta S.K, Habash R, Guasch A, Kalayjian R, Appel R, Fields T.A, Svetkey L.P, Flanagan K.H, Klotman P.E, et al., (2004).** The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney Int.* 66(3):1145-1152.

**Tait L, Soule H, Russo J, (1990).** Ultrastructural and immuno-cytochemical characterization of an immortalized human breast epithelial cell line MCF- 10. *Cancer Res.* 50: 6087-6094.

**Tanji N, Tanji K, Kambha M.N, et al., (2001).** Adefovir nephrotoxicity: Possible role of mitochondrial DNA depletion. *Hum Pathol* 32:734–740.

**Thompson M, (2011).** Liver, Kidney, Bone: Emerging Issues of Long Term Antiretroviral Therapy. 20th Annual HIV Conference of the Florida/Caribbean AIDS Education and Training Centre. May 13-14, 2011. Orlando, FL.

**Toffaletti J.G, Jones P. J, (1984).** Electrolytes. *In* Kaplan L.A and Pesce, A.J. (eds), 3<sup>rd</sup> ed. St. Louis: Mosby Publishers, pp. 272-292.

**UNAIDS, (2013).** UNAIDS report on the global AIDS epidemic.

**van der Merwe M.J, Jenkins K, Theron E, van der Walt B.J, (1993).** Interaction of the di-catechols rooperol and nordihydroguaiaretic acid with oxidative systems in the human blood: a structure activity relationship. *Biochem. Pharmacol.* 45: 303-311.

**van Wyk B.E, (2008).** A broad review of commercially important South African medicinal plants. *J. Ethnopharmacology* 119: 342-355.

**Walker U, Bauerle J, Laguno M, Murillas J, Mauss S, Schmutz G, Setzer B, Miquel R, Gatell J.M, Mallolas J, (2004).** Deletion of mitochondrial DNA in liver under antiretroviral therapy with didanosine, stavudine or zalcitabine. *Hepatology.* 39:311–317.

**Walter F, Boron D, (2004).** *Medical Physiology: A Cellular and Molecular Approach.* Elsevier/Saunders. ISBN 1-4160-2328-3.

**WHO and UNAIDS, (2015).** AIDS epidemic update.

**WHO, (2009).** A Rapid advice; antiretroviral therapy for HIV infection in adults and adolescents. Switzerland.

**Wilt T.J, Ishani A, MacDonald R, Stark G, Mulrow C.D, Lau J, (2011).** Beta-sitosterols for benign prostatic hyperplasia- review. John Wiley and sons, Ltd. Minnesota. Abstract.

**Winston J.A, Klotman M.E, Klotman P.E, (1999).** HIV-associated nephropathy is a late, not early, manifestation of HIV-1 infection. *Kidney Int.* 55(3):1036-1040.

**[www.ivyroses.com/HumanBody/.../Urinary\\_System\\_Kidney\\_Diagram](http://www.ivyroses.com/HumanBody/.../Urinary_System_Kidney_Diagram).**

[www.ivyroses.com/HumanBody/.../Urinary\\_System\\_Nepbron\\_Diagram](http://www.ivyroses.com/HumanBody/.../Urinary_System_Nepbron_Diagram).

**Wyatt C. M., Morgello S., Katz-Malamed R, (2009).** The spectrum of kidney disease in patients with AIDS in the era of antiretroviral therapy. *Kidney Int'l.* 75(4):428–434.

**Wyatt C.M, Arons R. R, Klotman P. E, Klotman M. E, (2006).** “Acute renal failure in hospitalized patients with HIV: risk factors and impact on in-hospital mortality”. *AIDS.* 20(4): 561–565.

**Zembower T.R, Gerzenshtein L, Coleman K, Palella Jr F. J, (2008).** “Severe rhabdomyolysis associated with raltegravir use”. *AIDS.* 22(11): 1382–1384.

**Zimmermann A.E, Pizzoferrato T, Bedford J, Morris A, Hoffman R, Braden G, (2006).** “Tenofovir-associated acute and chronic kidney disease: a case of multiple drug interactions”. *Clin Infect Dis.* 42(2): 283–290.