THE EFFECTS OF ATRIPLA AND ALCOHOL CO–ADMINISTRATION ON THE HISTOMORPHOMETRY OF THE LIVER AND KIDNEYS OF ADULT MALE SPRAGUE DAWLEY RATS.

A dissertation submitted to the Faculty of Health Sciences, University of Johannesburg, in fulfilment of the requirements for the degree of Master of Technology: Biomedical Technology

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Johannesburg, 2018

DECLARATION

I, Olukemi Funmilayo Daramola (Student Number: 201422762), declare that this study is my own original work. Where someone else's work was used, due acknowledgement was done and references done and reference was made according to the universities specifications. This work has not been submitted for any degree or examination in any other institution.

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Signed: OLUKEMI FUNMILAYO DARAMOLA

The _____ day of the _____ 2018

DEDICATION

This research is dedicated to Mr. Folarin Adegbenro Daramola, my beloved husband, for his total moral support, encouragement and belief in my career. Also, to my lovely children Mr Adefare Maxwel Daramola my eldest son and to my twins Miss Oriade Taye Daramola and Mr Adeori Kenny Daramola for their support, patience and co–operation.

ACKNOWLEDGEMENTS

- I give glory to God Almighty, the Alpha and the Omega, the author and the finisher of my faith. For his mercies endures forever, he whom by his grace has made it possible for me to complete my studies.
- I want to appreciate my supervisor Dr. P Nkomozepi for his total support, encouragement, time and dedication to make this work a success.
- To my co–supervisor Mr. N Xhakaza for his guidance, time, dedication and his faith in me that I will make it.
- To the Head of Biomedical Technology Department Mrs Julian Mthombeni for her time and support.
- To our able laboratory technician Mr Mandla Sibiya for his assistance, time and encouragement.
- To my mother–in–law Mrs. Deborah Atere for her prayers, help and support throughout the programme.
- To my parents Mr. Samuel Akanni Owoeye and Mrs Victoria Oluwayemisi Owoeye for their love, prayers and blessings.
- To Professor Mbajiorgu and Mr Trust Nyirenda for their major contribution in the experimental work.
- To the University of Johannesburg, for their financial support through the Supervisor linked bursary.
- To my colleagues Mrs. Grace Oyewole and Ms Nokwanda Ngcakaza for their help and support when I needed it.

ABSTRACT

Highly active antiretroviral therapy (HAART) has increased the life expectancy of HIV positive individuals. However, an increase in the incidence of liver and kidney diseases among patients receiving HAART has been noted. No studies have demonstrated the effect of concomitant alcohol and HAART intake on the histomorphometry of the liver and kidneys. This study investigated the effects of co-administration of alcohol and Atripla on the histomorphometry of the liver and kidneys of adult male Sprague Dawley rats. Findings from this study revealed hypertrophy of the hepatocytes in zone III, increases in hepatocyte nuclear size in zone I and zone III and vacuolization of cytoplasm in treatment groups. In the kidneys, increase in size of the renal corpuscle and the glomerular tuft, reduction in size of the epithelial cells in the proximal and distal convoluted tubules was observed in treatment groups. In conclusion, treatment with Atripla alone or Atripla with alcohol resulted into liver and kidney injury.

LIST OF ABBREVIATIONS

A	Area
$A_{fraction}$	Area fraction
AKI	Acute kidney injury
ALD	Alcoholic liver disease
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AP	Area per point
ARF	Acute renal failure
ART	Antiretroviral therapy
ARV	Antiretroviral
AST	Aspartate aminotransferase
ATN	Acute tubular necrosis
b.w.i.p	Body weight intraperitoneal
BUN	Blood urea nitrogen
CA	Cytoplasmic area
CAS	Central animal services
CKD	Chronic kidney disease
C_{max}	Maximum concentration
C_{min}	Minimum concentration
CT $A_{fraction}$	Connective tissue area fraction
DCTEA	Distal convoluted tubular epithelial area
DCTLA	Distal convoluted tubular luminal area
DCTOA	Distal convoluted tubular outer area
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DPX	Distyrene plasticizer xylene
E	Ethanol
EFV	Efavirenz
FDA	Food and drug administration

FDC	Fixed dose combination
FTC	Emtricitabine
GGT	Gamma – glutamyltransferase
GT CT $A_{fraction}$	Glomerular tuft connective tissue area fraction
GTA	Glomerular tuft area
H&E	Haematoxylin & eosin
HA	Hepatocyte area
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
IgA	Immunoglobulin alpha
IS CT $A_{fraction}$	Interstitial connective tissue area fraction
MRP4	Multidrug resistance associated protein 4
MT	Masson trichrome
NA	Nuclear area
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NAFLD	Nonalcoholic liver disease
NNRTIs	Non – nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
PAS	Periodic acid Schiff's
PCTEA	Proximal convoluted tubular epithelial area
PCTLA	Proximal convoluted tubular luminal area
PCTOA	Proximal convoluted tubular outer area
RCA	Renal corpuscular area
SREBP1	Sterol regulatory element – binding protein 1
TDF	Tenofovir disoproxil fumate
T_{max}	Time to maximum concentration
TNF α	Tumor necrosis factor alpha
TWS	Tap water substitute
USA	Urinary space area

LIST OF SYMBOLS

%	Percent
˚C	Degree centigrade
=	Equal
±	Plus or minus
÷	Division
∑p	Sum of the points
µM	Micromoles
µM/h	Micromoles per hour
cm^3	Cubic centimeter
g	Gram
h	Hour
kg	Kilogram
mg	Milligram
mm^2	Millimeter squared
X	Multiplication

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CHAPTER ONE INTRODUCTION

1.0 Background

The use of highly active antiretroviral therapy (HAART) has effectively reduced human immunodeficiency virus (HIV) related morbidity and mortality (Palios *et al*., 2011). However, an increase in the number of deaths of people receiving HAART medication due to the liver and kidney diseases has been reported (Weber *et al*., 2006; Winston *et al*., 2008). Highly active antiretroviral therapy medication has been associated with several adverse side effects including hyperlipidemia, hypertension, insulin resistance, non-alcoholic fatty liver disease and chronic kidney disease (Grinspoon and Carr, 2005; Tanaka *et al*., 2006; Guaraldi *et al*., 2010; Zarghani *et al*., 2016).

Depending on the particular combination of HAART medication used, approximately 30% of patients develop serious chemical liver damage at the onset of treatment (Sulkowski *et al*., 2000). Hypersensitivity reactions and lactic acidosis are among the acute clinical symptoms of HAART induced liver toxicity with possible long term liver complications including sudden hepatic failure (Núñez, 2010). In addition, HAART medication is also associated with the development of acute and chronic kidney diseases (Kalyesubula and Perazella, 2011). Roe *et al*. (2008) reported that antiretroviral related toxicity accounts for approximately 14% of acute kidney injury (AKI) cases that occur at the initiation of HAART. Furthermore, several studies have also linked HAART medications containing tenofovir diprovoxil fumarate (TDF) to increased serum creatinine levels and reduced glomerular filtration rates (Antoniou *et al*., 2005; Gallant *et al*., 2005; Jülg *et al*., 2005; Winston *et al*., 2008).

Atripla, an example of HAART medication, is a combination of Efavirenz, Emtricitabine and Tenofovir disoproxil fumate (TDF) used as a single pill taken once daily (Dave *et al*., 2011; Pavitt *et al*., 2015). Labarga *et al*. (2009) stated that up to 22% of HIV positive patients administered with TDF, a component of Atripla, show additional irregular variables used to determine kidney tubular function. However, the increased incidence of liver and kidney diseases among patients

receiving HAART can be attributed to the toxicity of HAART medications and concomitant intake of alcohol (Barve *et al*., 2010; Kalyesubula and Perazella, 2011; Schaeffner and Ritz, 2012). Several studies have reported a number of cases of concomitant use of HAART and alcohol (Miguez *et al*., 2003; Núnez, 2006; Barve *et al*., 2010). Given that alcohol intake on its own alters liver and kidney function, there is a high risk of increased toxicity following its concomitant use with HAART (Epstein, 1997; Bini *et al*., 2007). Many HAART medications go through crucial metabolic processing in the liver and there is a significant chance for alcohol to alter their biotransformation (Pandrea *et al*., 2010).

The breakdown of alcohol in the liver results in the generation of hazardous by-products such as acetaldehyde and highly reactive oxygen species which result in liver injury (Maher, 1997). Lim *et al*. (2008) reported an increased incidence of liver damage among individuals engaging in dangerous or debauch drinking. Studies have shown that excessive alcohol intake is associated with advanced liver and kidney diseases (Bini *et al*., 2007; Schaeffner and Ritz, 2012). In the kidney, excessive alcohol intake is associated with IgA glomerulonephritis and acute nephropathy (Schaeffner and Ritz, 2012). Van Thiel *et al*. (1977) reported alcohol induced renal complications including kidney swelling, enlarged cells, accumulation of fat and reduced kidney function in alcohol – fed rats. In addition, Chaikoff *et al*. (1948) reported abnormal thickening of the basement membrane and cell proliferation in the glomerulus; enlarged and altered cells in the renal tubules.

According to our knowledge, no information has been published on the effects of co-administration of alcohol and HAART on the histomorphometry of the liver and kidneys. The goal of the current study was therefore to investigate the effects of co-administration of Atripla and alcohol on the histomorphometry of liver and kidneys of adult male Sprague Dawley rats.

CHAPTER TWO LITERATURE REVIEW

2.0 Highly active antiretroviral therapy

Highly active antiretroviral therapy (HAART) is currently the most common treatment regimen of HIV infection (Ledergerber *et al*., 1999). The use of HAART has significantly reduced morbidity and mortality due to HIV initiation (Palios *et al*., 2011). Highly active antiretroviral therapy was introduced in 1996 in the Western countries with either non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors in combination with at least two nucleoside reverse transcriptase inhibitors (NRTIs) (Ives *et al*., 2001). Subsequently, HAART was introduced in South Africa in April 2004 (Boulle *et al*., 2008). The mixture of two or more active regimen modes such as HAART was developed to decrease the viral load in the blood of patients undergoing at least 6 months therapy (Carpenter *et al*., 1997). The first set of fixed-dose combination (FDC) tablet used to treat HIV infection in 1997 was Combivir™ a composition of two nucleoside regimens namely: zidovudine and lamivudine (Esté and Cihlar, 2010). There are 25 approved single antiretroviral drugs belonging to the following 6 mechanistic classes that can be used to model multiple composition regimens: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, entry/fusion inhibitors and maraviroc and integrase inhibitors (Masho *et al*., 2007; Esté and Cihlar, 2010). In South Africa, Atripla is used as the first line fixed-dose combination.

2.1 Atripla

Atripla is a combination of Emtricitabine (FTC) and Tenofovir disoproxil fumate (TDF) two NRTIs and Efavirenz (EFV) NNRTIs used as one pill taken once a day (Dave *et al*., 2011; Pavitt *et al*., 2015). Atripla was approved by the US Food and Drug Administration (FDA) in 2006 and is currently prescribed to almost 80% of HIV infected patients (Clay *et al*., 2008; Julg and Bogner, 2008; Hughes, 2009). The South African national Department of Health recommended Atripla as the standard first line drug regimen in 2011 (Iwuji *et al*., 2013). Atripla is the first example of FDC containing all elements used as preferred antiretroviral regimen (Bartlett and Lane, 2007; Clay *et al*., 2008).

2.1.1 Emtricitabine

Emtricitabine (FTC) is a potent deoxycytidine nucleoside reverse transcriptase inhibitor approved for use since 2003 by the FDA (Wang *et al*., 2004; Semvua and Kibiki, 2011). The chemical structural name of emtricitabine is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one (Figure 2.2) (Devunuri *et al*., 2016). The molecular formula is $C_8H_{10}N_3O_3SF$ and it has a molecular weight of 247.25g (Devunuri *et al*., 2016).

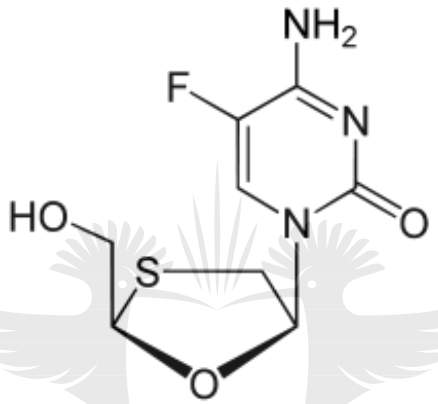

Figure 2.1. Chemical structure of emtricitabine. Adapted from Devunuri *et al*. (2016)

Emtricitabine is effective and well tolerated with other combined HAART regimens leading to reduction in the viral load of treated patients (Bang and Scott, 2003; Masho *et al*., 2007). Emtricitabine is taken orally at an approved dosage of 200mg daily and is quickly digested accompanied by high point plasma absorption that occurs within 1 – 2 hours (Sax, 2007; Clay *et al*., 2008). Saravolatz and Saag (2006) stated that emtricitabine has no known phase I glucuronidation or phase II cytochrome P450 enzymes in the liver. In addition, emtricitabine is completely eliminated through the kidneys and almost 86% of the dosage is eliminated in the urine with 13% of the dose excreted as metabolites (Sax, 2007; Clay *et al*., 2008). The inert substances of emtricitabine include 3′-sulfoxide diastereomers and their glucuronic acid conjugate (Clay *et al*., 2008). The biotransformation of emtricitabine includes oxidation of the thiol moiety to form the 3'-sulphoxide diastereomers approximately 9% of dose and conjugation with glucuronic acid to form 2'-O-glucuronide approximately 4% of dose (Clay *et al*., 2008). Emtricitabine did not

inhibit *in vitro* metabolism mediated by the following human CYP450 isoenzymes: 1A2, 2A6, 2B6, 2C9, 2C19, 2D6 and 3A4 also emtricitabine did not inhibit uridine-5′-diphosphoglucuronyl transferase, the enzyme responsible for glucuronidation (Clay *et al*., 2008).

Furthermore, emtricitabine has about 4% low protein binding, and 93% of mean absolute bioavailability (Clay *et al*., 2008). The intracellular half-life of emtricitabine-triphosphate is longer ranging from 39 hours, a duration which support the once daily dosage (Semvua and Kibiki, 2011).

The most commonly reported adverse effects of emtricitabine include: anxiety, cough, dyspepsia, fever, headache, nausea and weight loss (Clay *et al*., 2008; Plosker, 2013). More so, lactic acidosis, severe hepatomegaly with steatosis and lipodystrophy have also been reported in patients treated with emtricitabine (Clay *et al*., 2008; Reust, 2011). In addition to the above, patients receiving emtricitabine have also been reported to have skin discoloration or skin hyperpigmentation (Masho *et al*., 2007; Clay *et al*., 2008).

2.1.2 Tenofovir diprovoxil fumarate

Tenofovir diprovoxil fumarate (TDF) is a nucleoside/nucleotide analog of deoxyadenosine monophosphate, approved by FDA for use in 2001 (Lyseng-Williamson *et al*., 2005; Masho *et al*., 2007). The chemical structural name of TDF is Bis (isopropoxycarbonyloxymethyl) ester of (*R*)-9-(2-phosphonylmethoxypropyl) adenine (Figure 2.2) (De Clercq, 2001). Its molecular formula is $C_{23}H_{34}N_5O_{14}P$ and molecular weight of 635.32g (De Clercq, 2001).

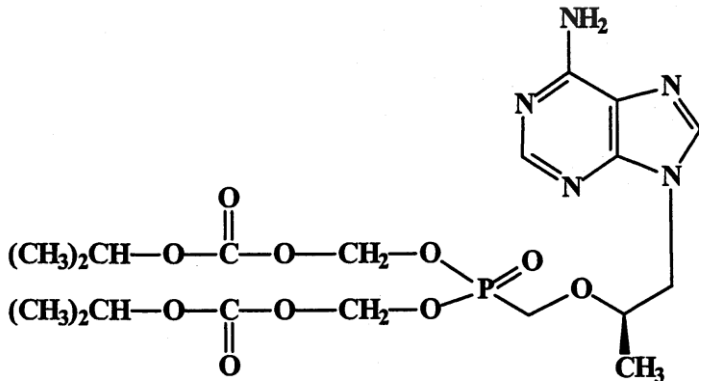

Figure 2.2: Chemical structure of tenofovir diprovoxil fumarate. Adapted from De Clercq (2001)

Tenofovir diprovoxil fumarate is hydrolyzed by diester prodrug to form tenofovir and phosphorylated by active metabolite to form tenofovir diphosphate (Lyseng-Williamson *et al*., 2005). Tenofovir diprovoxil fumarate is taken orally at a dose of 300mg once daily and approximately 70% - 80% of TDF is excreted unchanged in urine (Gallant and Deresinski, 2003; Plosker, 2013). Tenofovir diprovoxil fumarate is eliminated through glomerular filtration and tubular secretion (Gallant and Deresinski, 2003; Clay *et al*., 2008). Tenofovir diprovoxil fumarate has a long intracellular half-life, approximately 49 hours (Semvua and Kibiki, 2011).The most commonly reported adverse results in cases receiving TDF are equivalent to those of FTC which includes fear, headache, nausea and weight loss (Clay *et al*., 2008; Plosker, 2013).

2.1.3 Efavirenz

Efavirenz (EFV) is a non-nucleotide reverse transcriptase inhibitors, administered orally at a dose of 600mgand was approved for use by FDA in 1998 (Lichtenberg, 2003; *Clay et al*., 2008; Blas-García *et al*., 2010). Efavirenz is chemically described as (S)-6-chloro-4-cyclopropylethynyl-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one (Figure 2.3) (Ravikumar and Sridhar, 2009). The molecular formula is $C_{14}H_9ClF_3NO_2$ with molecular weight of 315.67g (Ravikumar and Sridhar, 2009).

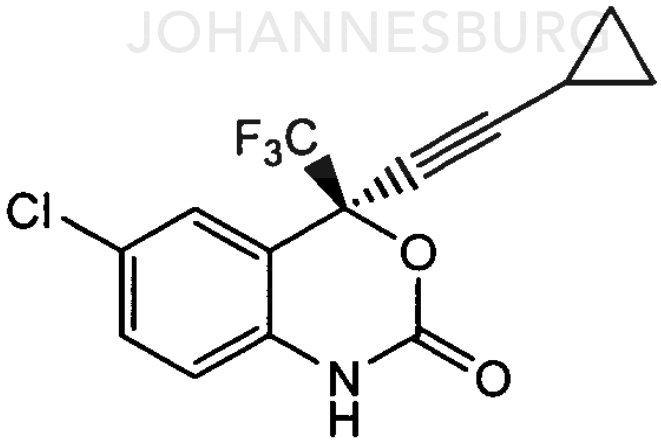

Figure 2.3: Chemical structure of efavirenz. Adapted from Ravikumar and Sridhar (2009)

Efavirenz is mainly metabolized by cytochrome P450s, such as CYP2B6 and CYP3A4 to hydroxylate metabolites which are eventually glucouronidated for excretion in the urine (Clay *et al.*, 2008; Semvua and Kibiki, 2011; Peter and Udoh, 2015). Efavirenz have the ability to enter into the central nervous system and spinal fluids with a capacity to strongly inhibit multi-drug resistant proteins such as MRP1, MRP2 and MRP6 (Peter and Udoh, 2015).

The oral bioavailability of EFV increases when administered with fatty meals (Semvua and Kibiki, 2011). Das *et al*. (1968) suggested that the oxidation of drugs and fatty acids might also be catalysed by nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) dependent mixed function oxidase system involving cytochrome P-450. About 14% - 24% of EFV is excreted in urine as the metabolites and 16%–61% of this drug is excreted in the feaces (Clay *et al*., 2008). Efavirenz has about 99% high protein binding which includes plasma proteins, such as albumin (Clay *et al*., 2008). The mean steady-state includes C_{max} values of 12.9 ± 3.7µM, C_{min} of 5.6 ± 3.2µM, AUC of 184 ± 73µM/h and T_{max} of 3-5h (Clay *et al*., 2008; Semvua and Kibiki, 2011). Efavirenz has a longer intracellular half-life from 40 – 55h (Semvua and Kibiki, 2011).

Efavirenz belongs to the group of NNRTIs which are allosteric inhibitors of the HIV-1 reverse transcriptase (Antonelli and Turriziani, 2012). Efavirenz bind in noncompetitive active mechanism to an allosteric pocket in the p66 subunit of the reverse transcriptase deforming the protein, thus obstructing the chemical step of polymerization (de Béthune, 2010; Das *et al*., 2012).

The adverse effects reported by patients using EFV medication are effects on central nervous system which include abnormal dreams, dizziness, headache, insomnia, agitation, amnesia, epilepsy and convulsions (Kontorinis and Dieterich, 2003; Gallego *et al*., 2004; Clifford *et al*., 2005; Jena *et al*., 2009). Other side effects of EFV include alcohol intolerance, fever, aches, pains and fatigue, fluid retention in the hands and feet, dry mouth, dyslipidemia, pancreatitis, skin problems, and asthma (Peter and Udoh, 2015).

2.2 Liver

The liver is the largest organ in the body with the exception of the skin, weighing approximately 1.5kg in adult about 2% of the adult weight (Mattson Porth, 2011; Sibulesky, 2013). It is located in the abdominal cavity inferior to the diaphgram (Mescher, 2011) . The liver is enclosed in a capsule of fibrous connective tissue called Glisson's capsule (Mattson Porth, 2011).

Histologically, the liver shows multiple hexagonal units called classical liver lobules (Figure 2.4) (Eroschencho, 2000)**.** Classical liver lobules are the functional units of the liver separated by connective tissue that contain bile ducts, lymphatics, nerves and blood vessels (Mescher, 2011). The portal areas are present at the corners of the lobules occupied by portal triads (Mattson Porth, 2011; Mescher, 2011). In humans, the liver contain 3 – 6 portal triads per lobule, individually accompanied by venule (a branch of the hepatic portal vein), an arteriole (a branch of the hepatic artery), a duct (part of the bile duct) (Mattson Porth, 2011; Mescher, 2011). In the middle of every classical lobule is the central vein (Eroschencho, 2000). Plates of liver cells called hepatocytes (basic structural components of the liver) radiate from the central vein (Eroschencho, 2000)**.** The hepatocytes are separated by the sinusoids that extend from the periphery of the lobules to the central vein (Mattson Porth, 2011). The hepatic sinusoids are unevenly dilated blood vessels lined by end layer of fenestrated endothelial cells (Eroschencho, 2000). Hepatic sinusoids detached out of the concealed hepatocytes by a subendothelial space called "space of Disse" that comprises microvilli of the hepatocytes (Mescher, 2011). The sinusoids receive blood from the portal vein and hepatic artery, as blood travel through the sinusoids, the hepatocytes are exposed to blood because the plates of the hepatic cells are no more than two layers thick (Mattson Porth, 2011). The sinusoids consists of two types of cells the endothelial and the reticuloendothelial cells called Kupffer cells (Mattson Porth, 2011). The main function of the Kupffer cells are to metabolize old red blood cells, digest haemoglobin, engulf bacteria and other foreign bodies from the portal blood as it pass through the sinusoids (Mattson Porth, 2011; Mescher, 2011).

The liver gets most of its blood from the portal vein, which carries oxygen-poor and nutrient-rich blood, and smaller portion of oxygenated blood from the hepatic artery (Mescher, 2011). In the liver the portal vein branches repeatedly and sends small portal venules called the interlobular

branches to the portal spaces (Mescher, 2011). The portal venules branch into smaller distributing venules that run around the periphery of each lobule and lead into the sinusoids, and the sinusoids run radially, converging in the center of the lobule to form the centrolobular vein (Mescher, 2011). The central vein increases in diameter as it receives more and more sinusoids (Mattson Porth, 2011). At the end, the central vein leaves the lobule base and merge with the larger sublobular veins which converge and fuse to become many large hepatic veins that drain into the inferior vena cava (Mescher, 2011).

The hepatic artery divides repeatedly into interlobular arterioles in the portal areas, few of them go straightly into the sinusoids at different interval from the portal spaces, thereby attaching oxygen-rich blood to the portal venous blood in the sinusoids (Mescher, 2011). Hepatocytes produce bile into small passages called bile canaliculi situated in the middle of each hepatocytes (Eroschencho, 2000). The canaliculi converge at the periphery of liver lobules in the portal areas as bile ducts (Eroschencho, 2000). The bile ducts then drain into larger hepatic ducts that carry bile out of the liver (Eroschencho, 2000). In the liver lobules, bile flows in bile canaliculi toward the bile duct in the portal area, whereas blood in the sinusoids flows toward the central vein (Eroschencho, 2000).

The liver acinus (of Rappaport), is a functional unit of liver parenchyma region irrigated by terminal branches of distributing veins (Mescher, 2011). Each acinus is diamond-shaped and is situated in adjacent areas of two classic liver lobules (Mescher, 2011). Liver acinar are grouped into the following three zones: the first zone is zone I that is also referred to as periportal zone, hepatocytes nearest to this zone receive the oxygen rich blood and nourishment (Mescher, 2011). Zone II is between zones I and III, hepatocytes around this area are indefinite and they receive less oxygen and nutrients and the hepatocytes around this zone have an intermediate range of metabolic functions (Mescher, 2011). Zone III is near the central vein, and is also referred to as perivenular zone, with the hepacytoctes around this zone receiving poor oxygen and nutrients (Mescher, 2011). Cells in Zone III are a site for glycolysis, lipid formation, drug biotransformations, fatty accumulation and susceptible to ischemic necrosis (Mescher, 2011).

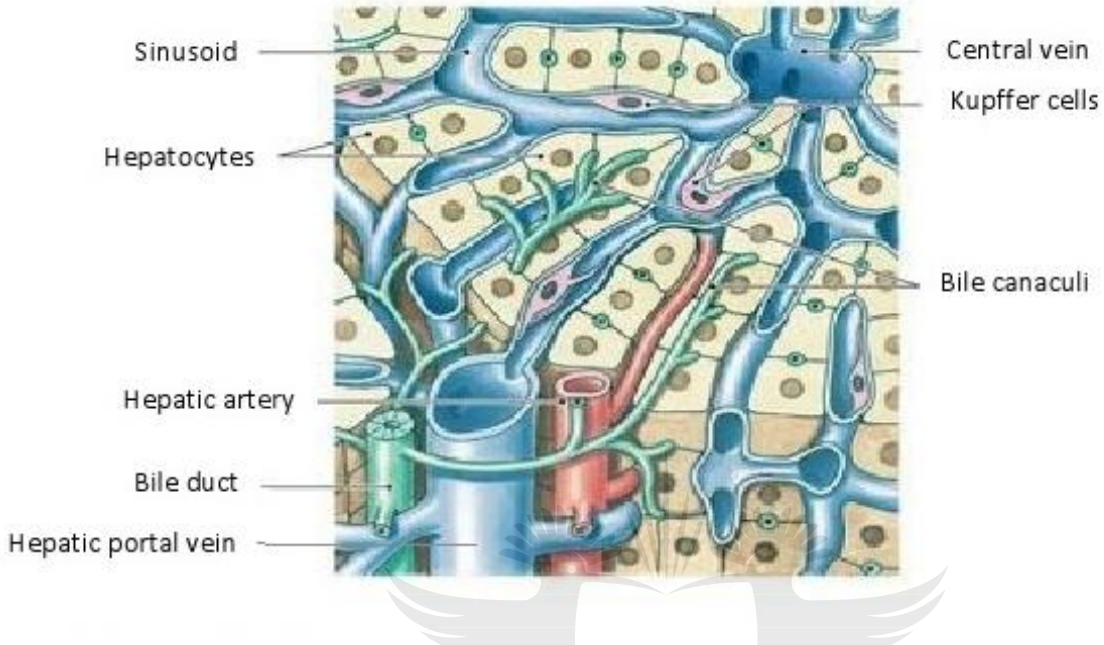

Figure 2.4: Schematic diagram showing the classical lobule of the liver. Adapted from Martini *et al*. (2009)

The function of the liver is collecting, changing, building up metabolites from blood, for counteracting and removal of toxic substances in blood through the bile (Mescher, 2011). Furthermore, the liver processes absorbed nutrients in the digestive tract and reserves it for use by other organs of the body (Mescher, 2011). In addition to the above, the liver also generates bile, metabolizes hormones and drugs, synthesizes plasma proteins such as albumin, fibrinogen, maintain blood glucose, stores minerals and vitamins, convert ammonia to urea and fatty acids to ketones, and remove bilirubin (Mattson Porth, 2011).

Blood tests are usually required to detect liver diseases and changes in liver function (Lee *et al*., 2010). The tests normally include serum levels of the liver enzymes that are used to diagnose the injury of the liver cells, ability of the liver to synthesize proteins and excretory functions of the liver (Mattson Porth, 2011). The serum liver enzymes include: alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum bilirubin, serum γ-glutamyltransferase (GGT) and

alkaline phosphatase (ALP) (Lee *et al*., 2010). The ALT and AST are used to measure the liver cells injury, plasma proteins and blood clotting factors while serum bilirubin, GGT and ALP are used to measure the hepatic excretory function (Mattson Porth, 2011). Increase in serum levels of liver enzymes usually indicates liver damage, liver injury or liver disease (Mattson Porth, 2011).

2.3 Kidneys

The kidneys are paired large bean-shaped organs located retroperitoneally against the posterior abdominal wall in the abdominal cavity on either side of the vertebral column (Eroschencho, 2000; Mattson Porth, 2011). Each kidney have a concave medial border called the hilum where blood vessels, nerves and lymphatic enter and the ureter exits (Mescher, 2011). These structures are surrounded by a loose connective tissue and a fat-filled space called the renal sinus and each kidney is covered by a fibrous connective tissue capsule (Eroschencho, 2000). Each kidney is divided into an outer cortex and an inner medulla, which consists of renal pyramids (Mescher, 2011). There are parallel arrays of tubules from the base of each medullary pyramids called the medullary rays which penetrate the cortex (Mescher, 2011). Every medullary rays contains many collecting tubules in conjunction with several nephrons (the functional units of kidneys) (Mescher, 2011). Around each medullary pyramid are a bunch of cortical tissue called renal lobe (Mescher, 2011). Each medullary ray is formed at the center of a conical renal lobule, and the cortical tissue is found in the middle of the medullary pyramids, these structure are referred to as columns of Bertin (Mattson Porth, 2011; Mescher, 2011). The round apex of each pyramid extends downward to the renal pelvis to form renal papilla and the portion of the cortex extends on each side of the renal pyramids to form the renal columns (Eroschencho, 2000). The renal papilla is surrounded by a funnel-shaped minor calyx, which collects urine from the papilla. The minor calyces join in the renal sinus to form a major calyx (Eroschencho, 2000). The major calyces join to form the larger funnel-shaped renal pelvis, which leaves each kidney through the hilum. The renal pelvis narrows down to become a muscular ureter which descends towards the bladder on each side of the posterior body wall (Eroschencho, 2000). The blood supply to the kidneys is via the renal arteries that enter the hilum of each kidney and divide into several segmental branches, which further branch into several interlobar arteries situated between the renal pyramids (Mescher, 2011). At corticomedullary junction, the interlobar arteries form the arcuate arteries that penetrates over the

base of the renal pyramids and become interlobular arteries (Eroschencho, 2000). The afferent arterioles arise from the interlobular arteries that supply blood into the capillaries in the glomeruli. These blood vessels leave the capillaries and enter the efferent arterioles which forms a peritubular capillary network and long straight capillary vessels called vasa recta (Eroschencho, 2000; Mescher, 2011). The vasa recta in the medulla loop back to the corticomedullary boundary and parallel to the loop of Henle (Eroschencho, 2000).

Microscopically each kidney consists of functional units called nephrons (Figure 2.5) (Eroschencho, 2000). Nephrons are of two types, cortical and juxtamedullary nephrons (Eroschencho, 2000). The cortical nephrons are found in the cortex of the kidneys, while the juxtamedullary nephrons are located at the junction of the cortex and medulla of the kidney (Eroschencho, 2000). The nephron is made up of renal corpuscle, proximal convoluted tubule, loop of Henle, distal convoluted tubule, collecting tubules and ducts (Mescher, 2011). Renal corpuscle comprises of a tuft of capillaries known as the glomerulus and are surrounded by a double layer of epithelial cells referred to as the glomerular or Bowman's capsule (Eroschencho, 2000; Mescher, 2011). The glomerulus are formed when the afferent arteriole enter the renal corpuscle, and divide into two or more primary branches and each is subdivided into capillaries (Mescher, 2011). The inside layer or visceral layer of the capsule contains epithelial cells called podocytes and the outside layer usually referred to as parietal layer of the glomerular capsule contains simple squamous epithelium (Eroschencho, 2000). The podocytes are not the only cells around the capillaries, there are other cells that are connected to the glomerulus, these cells are referred to as mesengial cells (Eroschencho, 2000). These cells serve as phagocytes that engulf any foreign bodies in the glomerular filter, produce extracellular matrix that provide structural support for the glomerular capillaries (Eroschencho, 2000). In the middle of the inner and outer layer of the glomerular is a urinary space that collect the filtered fluid from the capillary wall and the internal layer (Mescher, 2011).The renal corpuscle consists of vascular pole and urinary pole. The vascular pole is where the afferent arteriole comes in and the efferent arteriole exit and the urinary pole is where the proximal convoluted tubule starts (Mescher, 2011). The proximal convoluted tubules are located around the renal corpuscles in the cortex than the distal convoluted tubules because they are longer than the distal convoluted tubules (Eroschencho, 2000). The cells of the proximal convoluted tubules are large and contains three to five nuclei that are usually seen

at the center of the cell (Mescher, 2011). The cell border of the proximal convoluted tubules are not clearly defined due to extensive basal and lateral cell membrane interdigitating with the neighboring cells and the tubule also shows a small lumen, single layer of cuboidal cells with acidophilic granular cytoplasm because of many elongated mitochondria and a brush border (microvilli) that lines the cells (Eroschencho, 2000; Mescher, 2011). In the proximal convoluted tubules reabsorption of most of the substances from the glomerular filtrate takes place and as the glomerular filtrate enters the proximal convoluted tubules**,** all glucose, proteins, amino acids**,** carbohydrates, and about 75 to 85% of water, sodium and chloride ions are absorbed from the glomerular filtrate into peritubular capillaries (Eroschencho, 2000). The presence of microvilli on proximal convoluted tubule increase cells greatly at the surface area and facilitates absorption of filtered material (Eroschencho, 2000).

The loop of Henle has a U shaped structure that consist of a thick and thin descending limb and thick and thin ascending limb, the wall is lined with squamous epithelial cell which makes the lumen of the cell to be wide (Mescher, 2011). The distal convoluted tubules maintain their histological structure yet becomes twisted as the thick ascending limb of the loop of Henle that enters the cortex (Mescher, 2011). These tubules are not regularly seen in the cortex because they are smaller with few convolutions (Eroschencho, 2000). Histologically, the distal convoluted tubules have a simple cuboidal epithelial layer, smaller cells, no brush border (microvilli), more acidophilic, larger lumen, more cells, increased cellular interdigitations and the presence of elongated mitochondria within the infoldings (Eroschencho, 2000; Mescher, 2011). Reabsorption of sodium ions in the tubular filtrate is the main function of distal convoluted tubules, which are essential for the maintenance of acid base balance of body fluids and blood (Eroschencho, 2000). This reabsorption is influenced by the hormone called aldosterone, secreted by adrenal cortex (Eroschencho, 2000). Furthermore, small collecting tubules join together to form some larger collecting ducts (Mescher, 2011). These small collecting ducts are lined by lightly stained cuboidal epithelial cells and as the collecting ducts become larger, it moves towards the papillae of the medulla, referred to as papillary ducts Bellini (Eroschencho, 2000; Mescher, 2011). Deep down in the medulla**,** the cuboidal epithelial cells in the ducts changes to columnar, at the edge of each papilla, the papillary ducts empty their contents into the minor calyx (Eroschencho, 2000). The

area on the papilla that exhibits openings of the papillary ducts is referred to as the area cribrosa (Eroschencho, 2000).

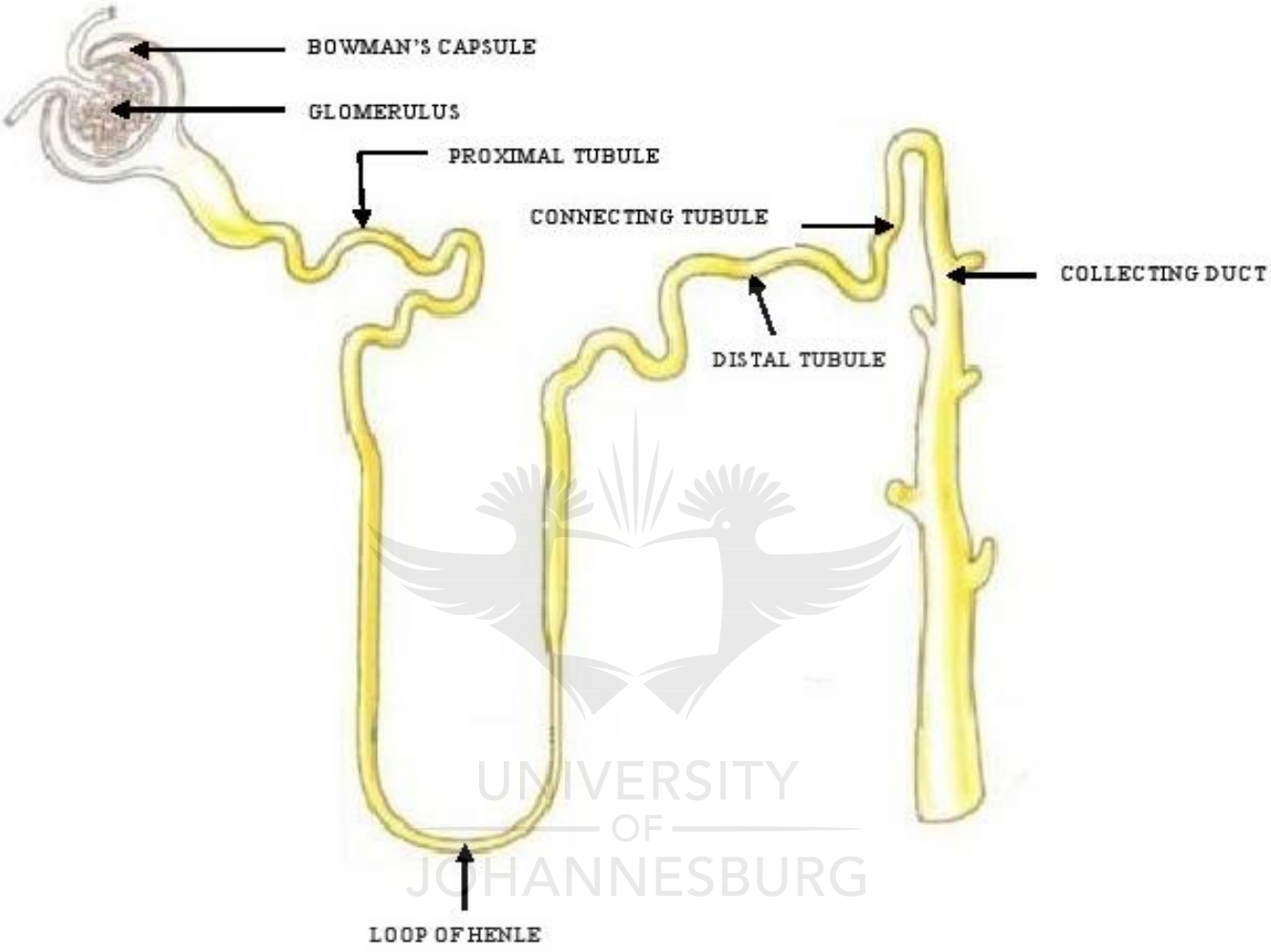

Figure 2.5: Structure of the nephron. Adapted from Eugenio-Pérez *et al*. (2016)

The main function of the kidneys is to control fluid and electrolytes balance in the body, removal of waste products and excess electrolytes, stimulate hormones such as renin and erythropoietin (Mattson Porth, 2011; Mescher, 2011). Blood tests are usually required to assess the function of the ability of the kidneys to remove metabolic wastes, retain electrolyte balance and to regulate body fluid as well as pH composition of the blood (Mattson Porth, 2011). The blood tests for

analyzing serum level of kidney function include sodium, potassium, uric acid, phosphate, calcium, bicarbonate, serum pH, blood urea nitrogen (BUN) and creatinine (Mattson Porth, 2011). Increase in serum levels of sodium, potassium, phosphate, BUN, uric acid and creatinine and decrease in serum levels of calcium, serum pH and bicarbonate indicate renal failure (Mattson Porth, 2011).

2.4 Effects of HAART and alcohol on the Liver

2.4.1 Effects of HAART on the liver

Hepatotoxicity is associated with the use of NNRTIs, one of HAART regimen combination in Atripla such as EFV (Palmon *et al*., 2002). Dieterich *et al*. (2004) reported that increased toxicity and other adverse effects are associated with HAART regimens combination which causes drug-induced liver injury, neuropathy, and pancreatitis. In addition to the above, it is reported that the use of efavirenz can lead to the development of hepatotoxicity (Sulkowski *et al*., 2002). However, according to Price and Thio (2010), although efavirenz can cause hepatotoxicity, nevirapine is more commonly linked to hepatotoxicity. Elevation of ALT and AST, the liver enzymes are correlated with hepatotoxic effects of HAART medications (Dieterich *et al*., 2004; Verma and Kaplowitz, 2009).

Highly active antiretroviral therapy has been reported to damage liver cells in different ways which include mitochondrial toxicity, accumulation of fat in the liver (Ogedegbe *et al*., 2003; Barve *et al*., 2010). Protease inhibitors one of the six classes of HAART regimens can also contribute to liver damage by causing injuries to the liver sinusoidal endothelial cells resulting to liver cirrhosis (Barve *et al*., 2010; Miyao *et al*., 2015; Greuter and Shah, 2016).

Nucleoside/nucleotide reverse transcriptase inhibitors have also been reported to cause hepatic steatosis by inhibiting mitochondrial deoxyribonucleic acid replication thereby resulting in accumulation of fat in the liver (Ogedegbe *et al*., 2003). In addition, other studies have indicated that treatment with HAART medication alters metabolic systems which is also associated with

the development of liver steatosis which can lead to inflammation and fibrosis (Barbaro, 2006; Ingiliz *et al*., 2009).

2.4.2 Effects of alcohol on the Liver

The use of alcohol has been grouped as non-dangerous and dangerous or debauch drinking (Lim *et al*., 2008; Chaudhry *et al*., 2009). One of the earliest pathological manifestations of alcohol misuse is the beginning of alcoholic fatty liver problem (Lieber, 1994). Neuman (2001) noted that cytokines also regulate apoptosis which is in part responsible for alcohol induced loss of liver tissue. Furthermore, Neuman (2001) reported that persistent cytokine secretion results in chronic inflammation which leads to conditions such as hepatitis, fibrosis and cirrhosis. Clinical changes related with alcohol consumption are steatosis, inflammation, necrosis, fibrosis, impairment of organ function and carcinogenesis (Gao and Bataller, 2011). Alcoholic liver diseases (ALD) include steatosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma (Gao and Bataller, 2011).

Alcohol is highly diffusible through cell membranes and is metabolized by most tissues, thus, its toxicity affects the liver because the liver is the major site of alcohol metabolism and it is targeted for alcohol-induced organ damage (Gao and Bataller, 2011). Liver cirrhosis account for about 16.6% cases of alcohol-attributable deaths worldwide (Organization, 2011). Alcohol-induced liver damage can be divided into three categories namely: alcoholic fatty liver, alcoholic hepatitis and alcoholic cirrhosis (French *et al*., 1993). Alcoholic fatty liver occurs as a result of the amount of fat deposition in the liver of heavy drinkers (Maher, 1997). On the other hand, alcoholic hepatitis occurs as a result of widespread inflammation and destruction of liver tissue in which scar liver tissue may start to replace healthy liver tissue, this process is known as fibrosis (Maher, 1997). Alcoholic cirrhosis is the most advanced form of liver disease, diagnosed in 15 to 30% of heavy drinkers (Dufour *et al*., 1993). Between 40 and 90% of deaths from cirrhosis are alcohol related (Dufour *et al*., 1993). Alcoholic cirrhosis of the liver is characterized by extensive fibrosis that stiffens blood vessels and distorts the internal structure of the liver, resulting in serious effective damage, that leads to subsidiarity defect of other organs, such as the brain and kidneys (Maher, 1997). Alcoholic cirrhosis is a terminal disease due to damage that occurs in the veins that carry the blood to the liver (Maher, 1997).

2.4.3 Effects of HAART and alcohol co–administration on the liver

Miguez *et al*. (2003) reported that about 25% of HAART patients are addicted to alcohol and continuous use of alcohol is related to non-response to HAART treatment. In addition, study have revealed that excessive use of alcohol is associated with severe hepatotoxicity in HAART patients (Núnez, 2006). However, alcohol together with other medications are processed through similar enzymes needed for bioconversion of the antiretroviral (ARV) remedies, therefore, alcoholic patients on HAART are at threat of drug reactions that may either reduce or elevate the effects of HAART (Kresina *et al*., 2002; Pandrea *et al*., 2010). Another potential consequence of drug– drug interactions are increased toxicity and development of liver damage in patients who already are at risk of developing liver disease because of the direct toxicity of alcohol. (Pandrea *et al.*, 2010). Barve *et al*. (2010) reported that alcohol and HAART have potential mechanisms that can instigate liver damage which include dysregulation of signaling molecules called cytokines and functional disorder of small cell component called proteasomes. In addition, excessive alcohol use and HAART can affect proteasome function (Barve *et al*., 2010). Alcohol and HAART medications alter the liver through cytokines and proteasomes in an overlapping fashion to produce hepatotoxicity (Barve *et al*., 2010).

Inordinate use of alcohol and HAART can alter proteasome function (Barve *et al*., 2010). Sterol regulatory element–binding protein (SREBP)1 assists to control the action of certain genes in a cell's DNA, that are usually destroyed by the proteasomes (Riddle *et al*., 2001). Accumulation of SREBP1 plays a crucial part in fat accumulation related with alcoholic and nonalcoholic fatty liver also, it takes part in the irregular accumulation of fat molecules in the liver of patients receiving HAART medication (Lemoine *et al*., 2006).

2.5 Effects of HAART and alcohol on the kidneys

2.5.1 Effects of HAART on the kidneys

The use of HAART has been associated with a number of toxicity, including those affecting the kidneys (Moreno-Cuerda *et al*., 2006). Kidneys play a crucial role in the metabolism and excretion

of HAART medication which expose the kidneys to various types of injuries including acute kidney injury (AKI), tubulopathies, chronic kidney disease (CKD) and nephrotoxicity (Kalyesubula and Perazella, 2011).

2.5.1.1 Nephrotoxicity

Studies have shown that TDF is associated with nephrotoxicity as well as TDF-induced renal toxicity in patients receiving HAART (Nelson *et al*., 2008; Reid *et al*., 2008; Crum-Cianflone *et al*., 2010). Tenofovir diprovoxil fumarate is absorbed by the proximal convoluted tubules and secreted into the lumen through multidrug resistance-associated protein-4 (MRP4) (Imaoka *et al*., 2006; Ray *et al*., 2006). Multidrug resistance protein-4 is responsible for transporting tenofovir into the tubular lumen (Izzedine *et al*., 2005c). Nephrotoxicity can be caused by influx and efflux imbalance of drugs in and out of the renal cells leading to an increase in the intracellular drug concentration (Izzedine *et al*., 2005c). Influx of tenofovir into renal tubular cells is facilitated by the human organic anion transporter 1 (Cihlar *et al*., 2001). A research done by Angel-Moreno-Maroto *et al*. (2006) reported that efavirenz can also cause renal toxicity due to hypersensitivity reaction which include pneumonitis, hepatitis, and interstitial nephritis.

2.5.1.2 Acute kidney injury (AKI)

Kalyesubula and Perazella (2011) reported that AKI is one of the common side effects of HAART medication. Rhabdomyolysis with pigment-related kidney injury are seen in patients receiving HAART medication who develop AKI (Joshi and Liu, 2000; Zembower *et al*., 2008; Dori *et al*., 2010). Peraldi *et al*. (1999) reported about 10% of AKI cases in patients receiving HAART medication were associated with myoglobinuric pigment nephropathy. In a meta-analysis study done by (Cooper *et al*., 2010), loss of kidney function as well as greater risk of AKI were observed in patients receiving HAART medication especially TDF.

2.5.1.3 Chronic kidney disease (CKD)

Chronic Kidney Disease is another side effect of HAART such as TDF (Atta *et al*., 2008). However, the prevalence of chronic kidney disease is difficult to measure in patients receiving HAART medication due to different screening techniques (Röling *et al*., 2006). Elevated creatinine level and proteinuria have been observed in about 7.2% to 32% of patients receiving HAART medication (Heilemariam *et al*., 2001; Szczech *et al*., 2002; Szczech *et al*., 2004).

2.5.1.4 Fanconi syndrome.

Fanconi syndrome can be described as generalized defect of membrane transporters in the proximal tubule, leading to renal loss of glucose, phosphate, calcium, uric acid, amino acids, bicarbonates and tubular proteins (Izzedine *et al*., 2003). The component of Atripla mostly associated with Fanconi syndrome is tenofovir with potential consequences of calcium and phosphorus dysregulation and osteomalacia (Earle *et al*., 2004; Izzedine *et al*., 2004; Peyrière *et al*., 2004). Fanconi syndrome in patients using HAART medication presents as severe tubular dysfunction with an elevation in creatinine levels, hypophosphatemia, and glycosuria (Izzedine *et al*., 2005a).

2.5.2 Effects of alcohol on the kidneys

Mohanachari *et al*. (1984) reported increase in aspartate aminotransferase, glutamine synthetase, glutamate dehydrogenase, succinate dehydrogenase, arginase and lactate dehydrogenase in the kidney of alcohol–fed rats. Excessive alcohol consumption alters renal absorption, metabolic process of folate and reduces renal tubular reabsorption (Ross and McMartin, 1996; Hamid and Kaur, 2006).

Alcohol dependence patients may encounter inadequate blood levels of electrolytes and serious damages in the body acid–base balance (Epstein, 1997). Too much intake of alcohol can damage the hormonal regulatory systems that control the kidney function by creating dangerous reactions such as reduced sodium and acute kidney failure (Epstein, 1997). Severe alcohol application in

rats changes renal sodium as well as potassium elimination (Assadi, 1989). In addition alcohol influences its reaction on the renal brush border membrane thereby generating structural reshape in the phospholipid bilayer that triggers sodium consumption (Elgavish and Elgavish, 1985).

Musabayane *et al*. (2000) described the effects of alcohol on the kidneys histologically and found that cortical parenchyma revealed disorganisation of the proximal tubules with disorientation of microvilli and luminal casts. Furthermore, proximal tubule cells exhibited partial degeneration as evidenced by the reduced height of the cells and the presence of a prominent luminal area containing cellular debris (Musabayane *et al*., 2000). The proximal tubule cells exhibited a large number of dense bodies in their cytoplasm while distal tubule cells exhibited degeneration of their apical cytoplasm (Musabayane *et al*., 2000).

2.6 Aims and objectives of the study

2.6.1 Aim of the study

To determine the effects of Atripla and alcohol co–administration on the histomorphometry of the liver and kidneys of adult male Sprague Dawley rats.

2.6.2 Objectives

I. To determine the effects of oral administration of Atripla on the histomorphometry of the liver and kidneys of Sprague Dawley rats using Haematoxylin and eosin (H&E), Masson's trichrome (MT) and Periodic acid Schiff's (PAS) reaction histochemistry.

II. To determine the effects of oral administration of alcohol on the histomorphometry of the liver and kidneys of Sprague Dawley rats using H&E, MT and PAS histochemistry.

III. To determine the effects of Atripla and alcohol co-administration on the histomorphometry of the liver and kidneys of Sprague Dawley rats using H&E, MT and PAS histochemistry.

CHAPTER THREE METHODOLOGY

3.1 Experimental animals

Forty adult male Sprague Dawley rats with ages between 11 and 12 weeks old (Lanning *et al*., 2002) were used in this study. The rats were acquired from the Central Animal Services (CAS) at the University of the Witwatersrand. The rats were housed individually in perspex cages with wood shavings as bedding. This is done according to Wits Animal housing protocol in order to monitor the feeding and health status of each rat. The individually house rats were then organized accordingly into their respective treatment study groups. A 12–hour light / 12-hour dark regimen was maintained throughout the study (Ajibola *et al*., 2013). Ambient temperatures of 24˚C ± 2˚C were maintained with adequate ventilation provided (Ajibola *et al*., 2013).

3.2 Experimental design

The rats were randomly assigned to four treatment groups with ten rats each as follows: Group one: served as control and received distilled water and plain gelatin, Group two: received Atripla only, Group three: received ethanol only, and Group four: received both Atripla and ethanol. All animals were allowed one week acclimatization before the commencement of treatments. Thereafter, all treatments were carried out for a period of 90 days (Co-operation and Development, 1998) (See also figure 3.1).

Atripla (14.93mg/kg body weight per rat) was administered using flavored gelatin cubes (1cm^3) once a day orally for 90 days to Atripla and ethanol groups (Deeks and Perry, 2010). This dose was adjusted from the human therapeutic dose to obtain the corresponding therapeutic doses for the rat model. Ethanol (6%) in distilled drinking water was provided *ad libitum* to combined group of ethanol and Atripla and the ethanol only groups (Mbajiorgu *et al*., 2009). Distilled drinking water, being the vehicle for ethanol, was given to the groups on Atripla treatment only. Plain flavoured gelatin cubes (1cm^3), the vehicle for Atripla, equivalent to volume of Atripla, was given to the control and ethanol groups. The rats were weighed before and after the experiment. At the end of the experiment, the rats were deeply anaesthetized with sodium pentobarbitone (200 mg/kg

b.w i.p) and sacrificed. Liver and kidneys were harvested, weighed and fixed in 10% formalin for 24 hours.

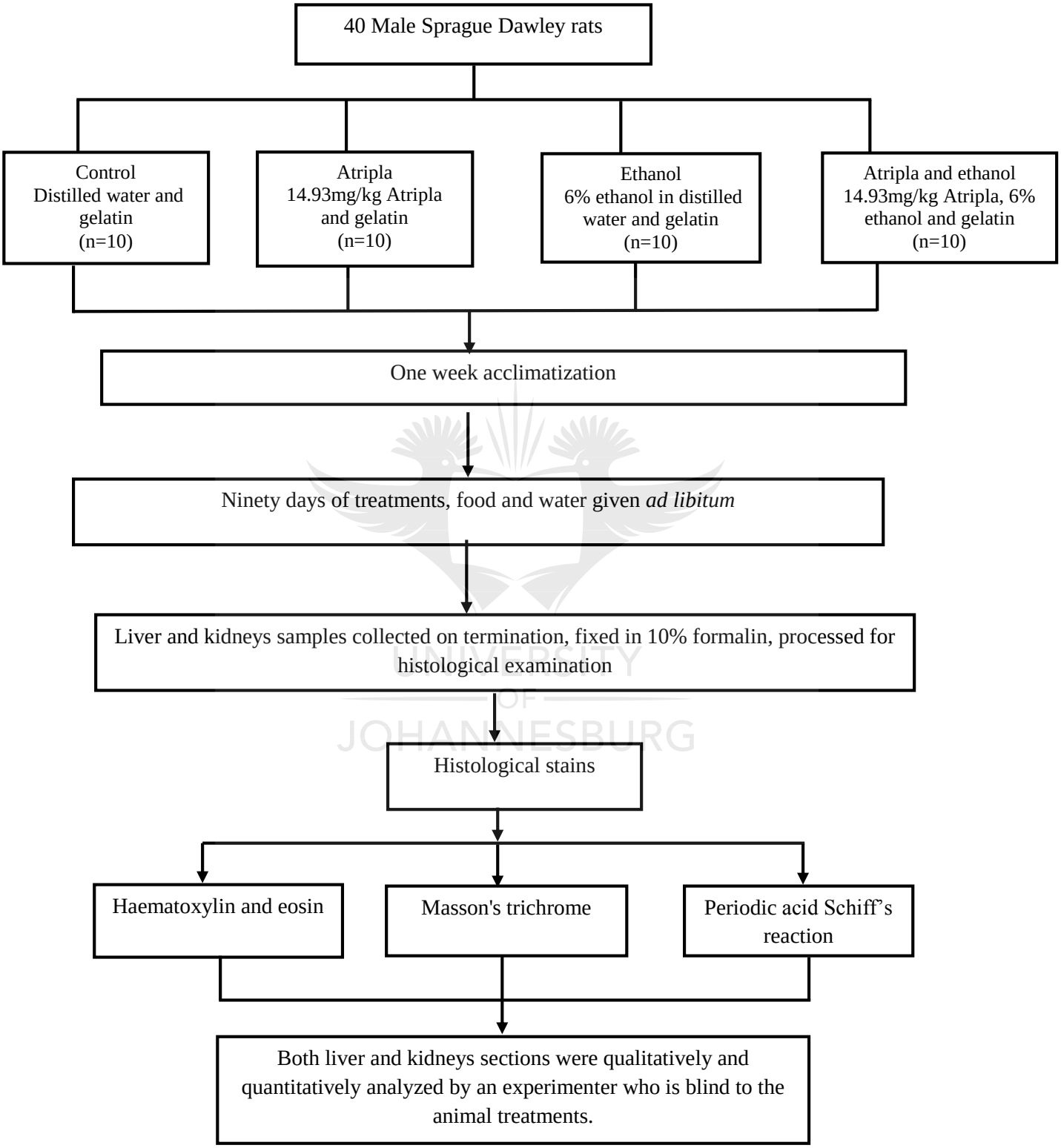

Figure 3.1: Schematic diagram showing the summary of the study design

3.3 Specimen collection, processing and staining

After fixation, the liver and kidneys were processed using the automatic tissue processor and embedded in paraffin wax. Sections of the liver and kidneys were cut at 3µm thickness using the rotary microtome and then mounted on glass slides before staining. The slides were stained with H&E, MT and PAS (Bancroft and Gamble, 2008). Haematoxylin and eosin was used for tissue architecture, Masson's trichrome for connective tissue and Periodic acid Schiff's reaction for glycogen.

3.3.1 Haematoxylin and eosin regressive staining procedure

The liver and kidneys sectioned sections were dewaxed twice in xylene for 5 minutes each. With agitation, the sections were hydrated through 100% ethanol for 30 seconds, 95% for 30 seconds, washed well in tap water and stained in Gill's haematoxylin for 20 minutes before they were washed well in water. The sections were then differentiated with acid alcohol for 15 seconds, washed immediately in tap water and examined under light microscope to ensure that the cytoplasm and other tissue elements were not stained except only the nuclei. After the differentiation, the sections were blued in Scott's tap water substitute for 30 seconds, washed well in tap water for 2 minutes before staining in 1% eosin for 5 minutes. The sections were then rapidly washed in tap water, dehydrated through 95%, 100% ethanol and cleared in xylene with agitation for 30 seconds. The slides were then placed in mounting xylene and mounted with distyrene plasticizer xylene (DPX) mounting media and cover slipped.

3.3.2 Masson's trichrome staining procedure

The liver and the kidney sections were dewaxed in two xylenes for 5 minutes each. With agitation, the tissues were hydrated through 100% ethanol for 30 seconds, 95% for 30 seconds, rinsed in distilled water and stained in stable iron haematoxylin for 5 minutes before being washed well in water. The sections were then differentiated microscopically with acid alcohol for 15 seconds, washed well in tap water, stained with Ponceau fuchsin for 10 minutes, rinsed with 1% acetic acid, treated with 1% phosphomolybdic acid for 2 and a half minutes, rinsed with 1% acetic acid, stained

with light green stain for 2 and a half minutes, rinsed with 1% acetic acid and blot dried. Tissues were dehydrated by dipping the slides rapidly through 100% ethanol and cleared in xylene with agitation for 30 seconds. The sections were then placed in mounting xylene, mounted with DPX and cover slipped.

3.3.3 Periodic acid Schiff's reaction

The liver and the kidney sectioned tissues were dewaxed in xylene 1 & 2 for 5 minutes each. With agitation, the tissues were hydrated through 100% ethanol for 30 seconds and 95% for 30 seconds, washed in distilled water and oxidized with 1% periodic acid for 5 minutes, and washed with distilled water. The sections were placed in a coplin jar with Schiff's reagent for 15 minutes, washed in running tap water for 10 minutes to remove uncombined leucofuchsin and stained in Gill's haematoxylin for 5 minutes. The sections were washed in running tap water for 2 minutes, blued with Scott's TWS for 30 seconds, washed in tap water, dehydrated rapidly through 100% ethanol and cleared in xylene with agitation for 30 seconds. The sections were then placed in mounting xylene, mounted with DPX and cover slipped.

3.4 Analyses

Both liver and kidney sections were qualitatively and quantitatively analyzed by an experimenter who was blinded to the animal treatments.

3.4.1 Qualitative analysis

Liver sections stained with H&E were assessed for cytoplasmic vacuolation, sinusoidal dilation and Kupffer cell activation according to Arsad *et al*. (2014). In addition, kidney sections stained with H&E were also assessed for the presence of pyknotic cells, granular casts, cellular casts, protein casts and hydropic degeneration according to Arsad *et al*. (2014).

High resolution microscopic representative photomicrographs for each treatment group were digitally captured using an ICC50 HD digital camera (Leica Biosystems, Germany) on a Leica

DM500 (Leica Biosystems, Germany) compound microscope connected to a PC running Leica application suite (LAS EZ; Leica Biosystems, Germany) software. Composite images were prepared with the Adobe Photoshop 7.0 software (Adobe Systems, Mountain View, CA). No pixelation adjustment or manipulation of the captured images was undertaken, except for the adjustment of contrast and brightness.

3.4.2 Quantitative analysis

From the liver sections, hepatocyte areas (HA) and nuclear areas (NA) for hepatocytes (H&E at 100X magnification) in the periportal (Zone I) and perivenular zones (Zone III) were measured using the ImageJ area tool (Schneider *et al*., 2012) . For each hepatocyte, the cytoplasmic area (CA) was determined by subtracting the nuclear area from the hepatocyte area (CA=HA minus NA). The interstitial or Zone II was excluded due to difficulty in defining its boundaries. In each liver, at least 20 hepatocytes were measured per each animal liver sections. In addition, the area (A) and area fraction ($A_{fraction}$) of the liver section occupied by connective tissue were also measured in zone I and zone III (MT at 40X) using the point counting method on ImageJ [A= ap X ∑p, where ap is the area per point and ∑p is the sum of the points falling on the connective tissue within a camera field (0.0816 mm²) of each liver section and $A_{fraction}$ = A÷ 0.0816 mm² X 100]. A total of 20 camera fields were used for each section.

From the kidney sections (PAS at 40X), renal corpuscular area (RCA) and glomerular tuft area (GTA) were measured using the ImageJ area tool (Schneider *et al*., 2012). For each renal corpuscle, the urinary space area (USA) was determined by subtracting the glomerular tuft area from the renal corpuscular area (USA= RCA minus GTA). In addition, the proximal convoluted tubular outer area (PCT OA) and proximal convoluted tubular luminal area (PCT LA) were measured using the area tool of ImageJ. The proximal convoluted tubular epithelial area (PCT EA) was determined by subtracting the proximal convoluted tubular luminal area from the proximal convoluted tubular outer area (EA= OA minus LA). Similarly, distal convoluted tubular outer area (DCT OA), distal convoluted tubular luminal area (DCT LA) and distal convoluted tubular epithelial area (DCT EA) were determined using the same method as described above for the PCT. In each kidney, at least 20 renal corpuscles, proximal convoluted tubules and distal convoluted

tubules were measured per each animal kidney section. Furthermore, the area (A) and area fraction ($A_{fraction}$) of the glomerulus tuft and renal interstitium occupied by connective tissue were also measured (MT at 40X) using the point counting method on ImageJ as described above. In each case, a total of 20 camera fields were used for each section.

3.5 Data analysis

All statistical analyses were done using Graphpad prism software for windows (Version 7.0). Measurements for each variable were expressed as mean ±standard deviation. Group means for each variable were compared using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test for *post hoc* analysis. A significance level of $P = 0.05$ was used.

CHAPTER FOUR RESULTS

4.1 Body, liver and kidney weights

In this experiment, animals with approximately the same body weights were used (*F*= 0.28; *P*= 0.84), (Table 4.1). However, at the end of the experiment, the animals treated with ethanol only, Atripla only and Atripla with ethanol had lower body weights compared to the control animals although the difference was not statistically significant *(F*= 0.78; *P*= 0.52), (Table 4.1). Consequently, body weight gain was higher in the control animals compared to the rest of the treatment groups even though the differences were not statistically significant (*F*= 1.28; *P*= 0.30), (Table 4.1).

Although not significantly different, liver weights were higher in the animals that received ethanol only, Atripla only and Atripla with ethanol compared to the control animals (*F*= 0.66; *P*= 0.58), (Table 4.1). Similarly, kidney weights were higher in the treatment groups compared to the control group even though the differences were not significant (*F*= 1.24; *P*= 0.31), (Table 4.1).

Table 4.1: Body, liver and kidney weights

	Treatment			
	Control	Ethanol	Atripla	Atripla+Ethanol
Initial body weight	465.50±37.15[a]	470.30±57.15[a]	474.00±49.27[a]	452.00±58.38[a]
Final body weight	636.90±31.14[a]	609.8±73.50[a]	627.30±47.09[a]	599.50±55.50[a]
Body weight gain	171.40±24.28[a]	139.50±54.58[a]	153.30±27.71[a]	147.50±16.27[a]
Liver weight	21.06±1.55[a]	22.95±3.62[a]	22.03±1.79[a]	22.20±3.26[a]
Kidney weight	1.74±0.19[a]	1.91±0.23[a]	1.89±0.15[a]	1.80±0.24[a]

Data of all variables expressed as mean ±standard deviation. Different superscripts indicate groups with significant differences. P <0.05

4.2 Liver

4.2.1 General liver histology

In H&E stained sections, liver sections of control animals exhibited normal typical hepatocellular architecture with central vein in the centre of each classic liver lobule, cords of hepatocytes separated by sinusoids radiating from it (Figure 4.1 A). The outlines of hepatocytes and sinusoidal spaces were clearly seen with no obvious histomorphological distortions. The other treatment groups exhibited varying degrees of distortion (Figures 4.1 B, C and D). Both zones I and III of the livers from the ethanol only group were characterized by distortion of the radial arrangement of hepatocytes, infiltration of inflammatory cells and dilation of sinusoids. In addition, the zone I region was characterized by atrophy of hepatocytes. On the contrary, zone III hepatocytes were hypertrophied and exhibited cytoplasmic vacuolation. Similar but more severe changes were also observed in the Atripla only treatment group. Although similar changes were observed in the Atripa with ethanol group, they were less severe than either the ethanol only or Atripla only groups. In MT stained sections, liver sections of all treatment groups except for the control were characterized by excessive accumulation of connective tissue fibres particularly in the periportal areas (Figure 4.2).

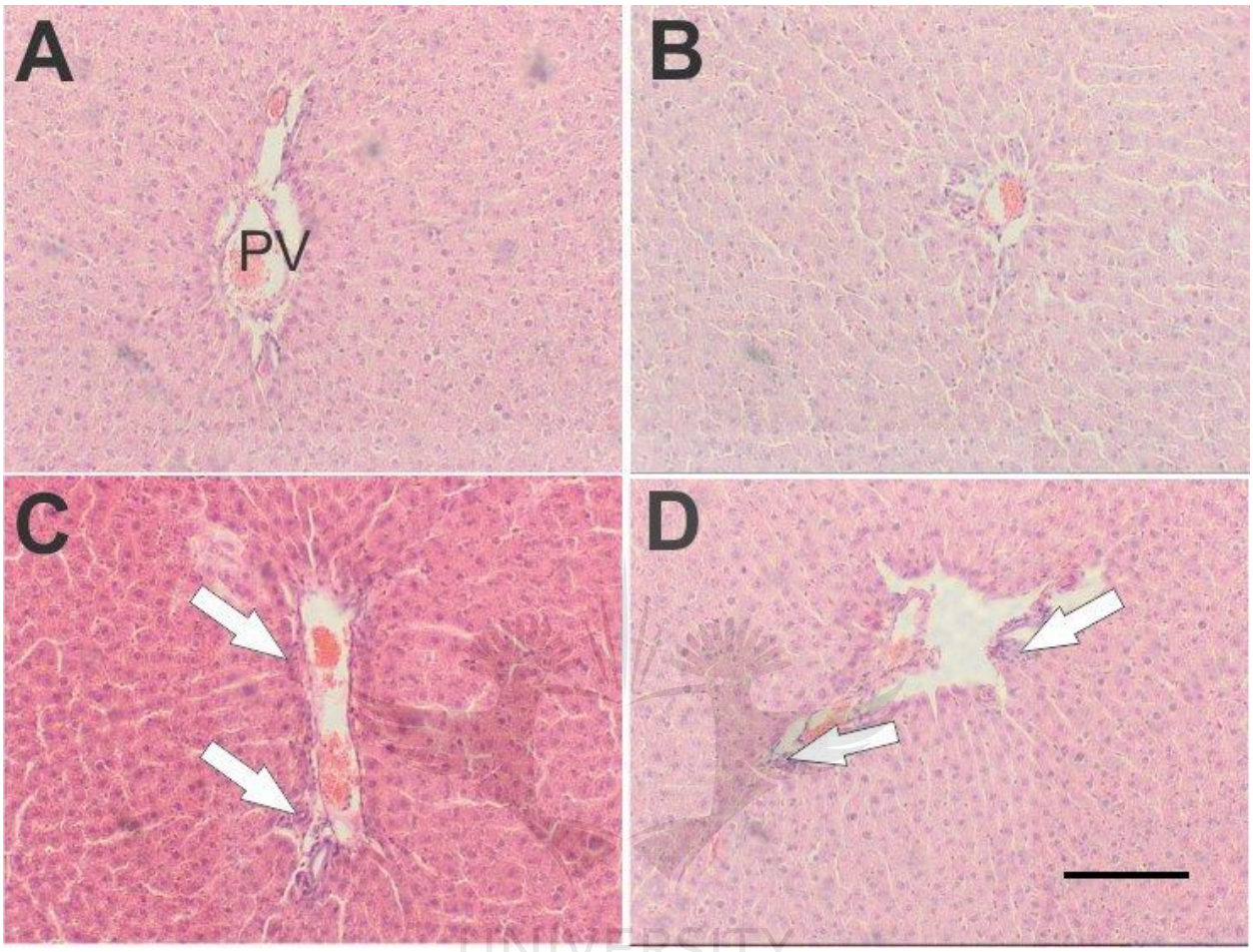

Figure 4.1: Photomicrographs showing changes in the architecture of the periportal region (Zone I) of the liver (H&E, 40X). A: Control, B: Ethanol only, C: Atripla only and D: Atripla with ethanol. Arrows indicate infiltration of inflammatory cells. Scale Bar in D = 20µm and applies to A, B and C. PV= Portal vein.

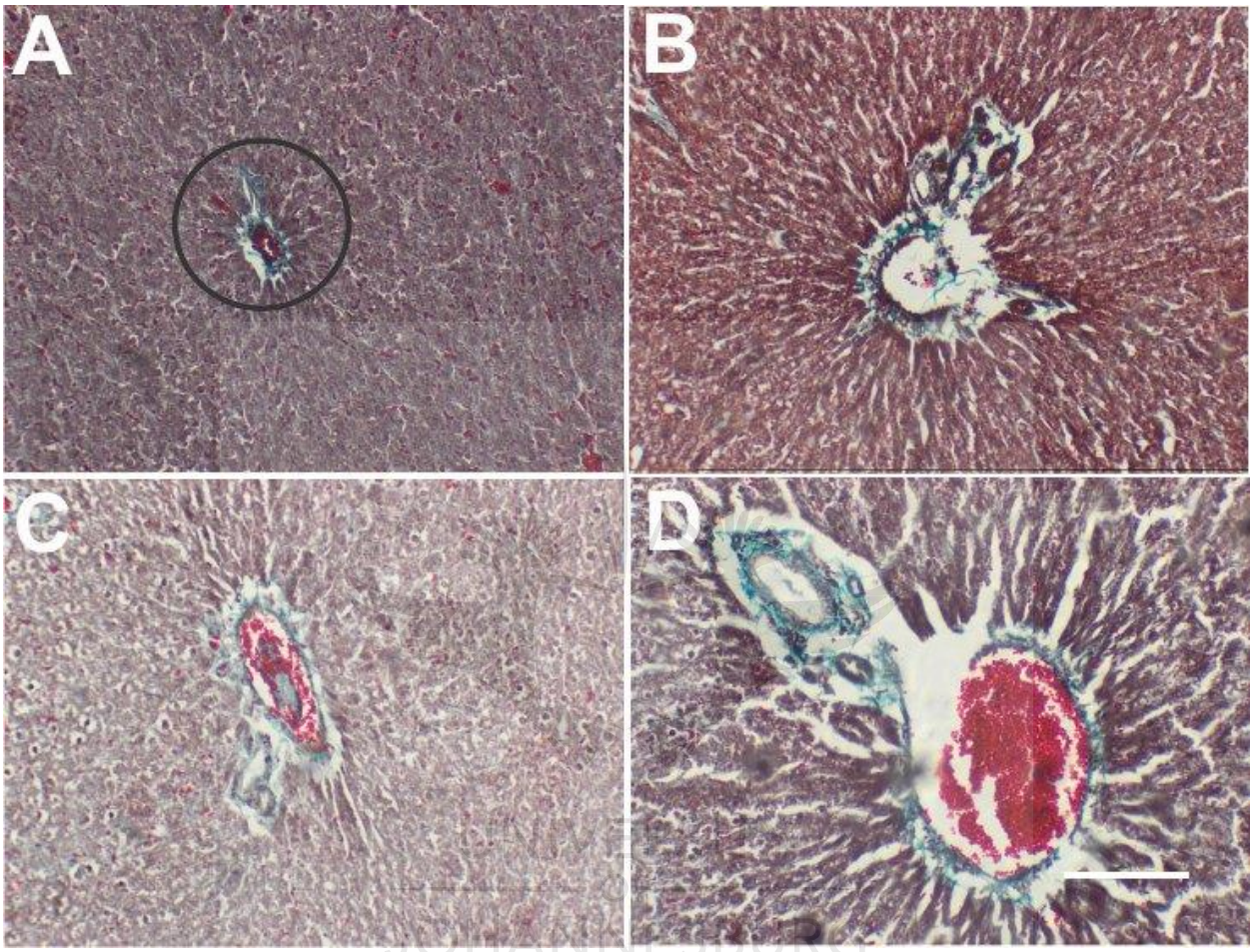

Figure 4.2: Photomicrographs showing the extent of fibrosis in the periportal region (encircled) of the liver (MT, 40X). A: Control, B: Ethanol only, C: Atripla only and D: Atripla with ethanol. Scale Bar in D = 20µm and applies to A, B and C.

4.2.2 Hepatocellular, nuclear and cytoplasmic areas in liver zone I

In zone I, no statistically significant differences were observed in the hepatocyte areas across all the treatments (F= 0.54; P= 0.65), (Table 4.2). However, nuclear area was 8%, 12% and 11% higher in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control groups (F= 3.71; P= 0.01), (Table 4.2). On the contrary, the cytoplasmic area was 5%, 8% and 9% lower in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control animals (F= 4.27; P= 0.01), (Table 4.2).

4.2.3 Hepatocellular, nuclear and cytoplasmic areas in liver zone III

In zone III, statistically significant differences were observed in the hepatocellular areas in the treatments groups compared to the control group (F= 2.84; P= 0.04), (Table 4.2). The hepatocellular area was 8%, 6% and 4% higher in the animals that received ethanol only, Atripla only and Atripla with ethanol respectively compared to the control animals (F= 2.84; P= 0.04), (Table 4.2). Likewise, the nuclear area was 16%, 14% and 11% higher in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control groups (F= 8.89; P= 0.0001), (Table 4.2). In addition, the cytoplasmic area was 9%, 7% and 6% higher in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control groups (F= 3.22; P= 0.02), (Table 4.2).

4.2.4 Connective tissue area fraction in the liver

The connective tissue area fraction in the liver was 33%, 100% and 66% higher in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control group (F= 9.56; P= 0.0001), (Table 4.2).

Table 4.2: Hepatocellular, nuclear, cytoplasmic areas and connective area fraction

		Treatment			
		Control	Ethanol	Atripla	Atripla+Ethanol
Zone I	HA	422.10±107.00[a]	417.10±105.30[a]	407.40±102.40[a]	414.70±103.30[a]
	NA	52.27±16.08[a]	56.47±17.65[a]	58.59±22.46[b]	57.95±17.92[b]
	CA	380.90±93.89[a]	360.70±100.40[a]	348.90±92.95[b]	347.20±81.83[b]
Zone III	HA	381.80±82.51[a]	410.60±104.80[b]	405.40±92.47[a]	395.70±98.48[a]
	NA	48.96±9.23[a]	56.61±14.18[b]	55.82±15.75[b]	54.24±16.34[b]
	CA	326.00±73.76[a]	353.90±99.80[b]	350.40±86.31[a]	347.70±89.47[a]
Liver	CT $A_{fraction}$	0.03±0.01[a]	0.04±0.02[b]	0.06±0.04[c]	0.05±0.03[d]

Zone I and III: HA=Hepatocellular area, NA= Nuclear area, CA= Cytoplasmic area, CT $A_{fraction=}$ Connective area fraction. Data of all variables expressed as mean ±standard deviation. Different superscripts indicate groups with significant differences. $P < 0.05$

4.3 Kidneys

4.3.1 General kidney histology

In H&E stained sections, kidney sections from control animals exhibited normal renal corpuscular, glomerular and tubular architecture in renal cortex of control animals (Figures 4.3 A). The rest of the treatment groups exhibited varying degrees of changes in the corpuscular, glomerular (Figures 4.3 B, C and D) and tubular changes. Kidney sections from the ethanol only and the Atripla only group were characterized by enlargement of the renal corpuscles, glomerular hypertrophy, mesangial expansion, infiltration of inflammatory cells, proximal and distal convoluted tubular dilatation. The Atripla with ethanol group was also characterized by severe infiltration of inflammatory cells and tubular dilation. However, this group was characterised by both renal corpuscular and glomerular atrophy. In MT stained sections, all treatment animals except the control were characterized by increase in glomerular and interstitial connective tissue fibres (Figure 4.4).

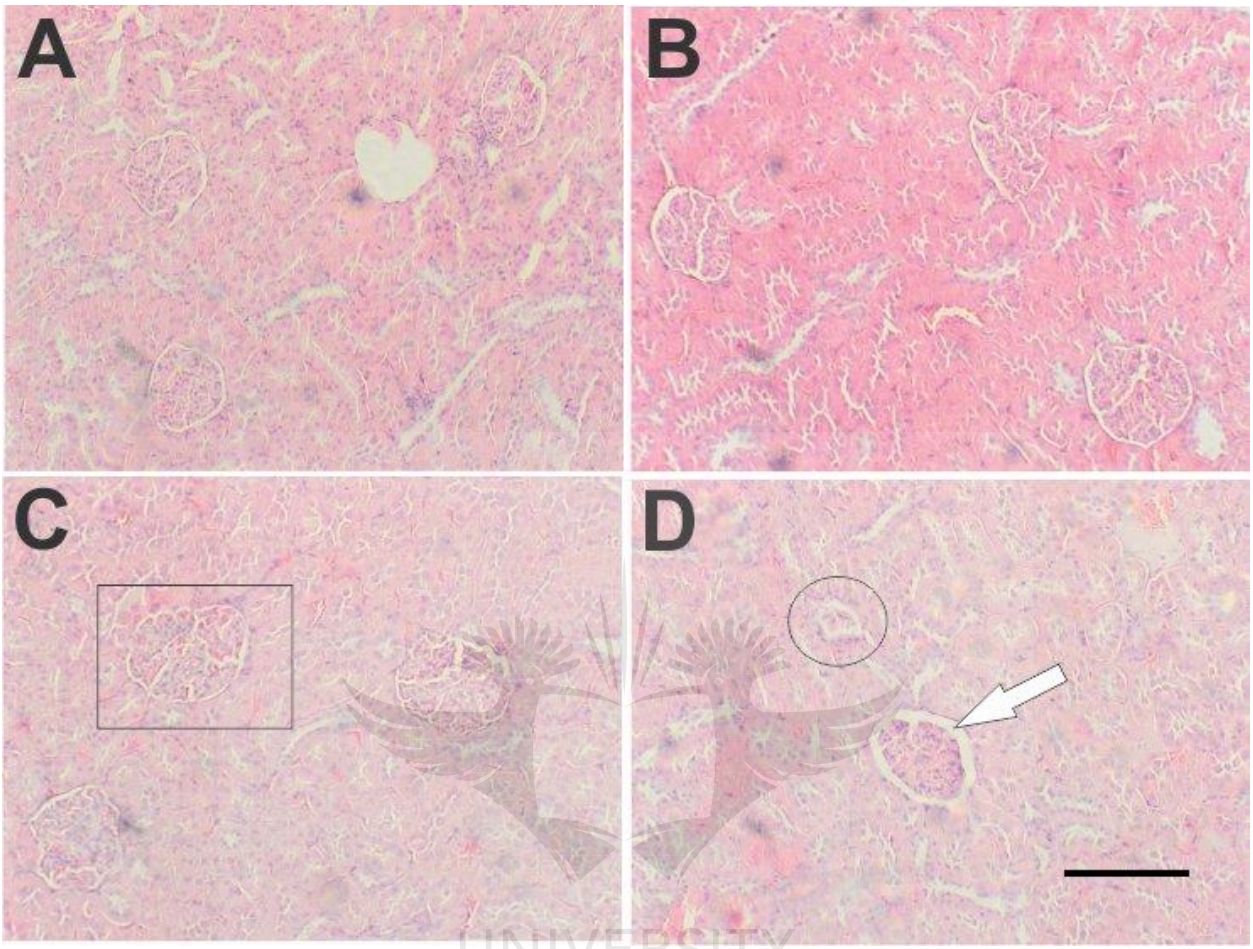

Figure 4.3: Photomicrographs showing the changes in the architecture of the cortical region of the kidney (H&E, 40X). A: Control, B: Ethanol only, C: Atripla only and D: Atripla with ethanol. Arrows indicate infiltration of inflammatory cells. The rectangle in C shows renal corpuscular hypertrophy. The encircled area in D shows renal corpuscular atrophy. The arrow in D shows dilatation of the urinary space as a result of glomerular tuft shrinkage. Scale Bar in D = 20µm and applies to A, B and C.

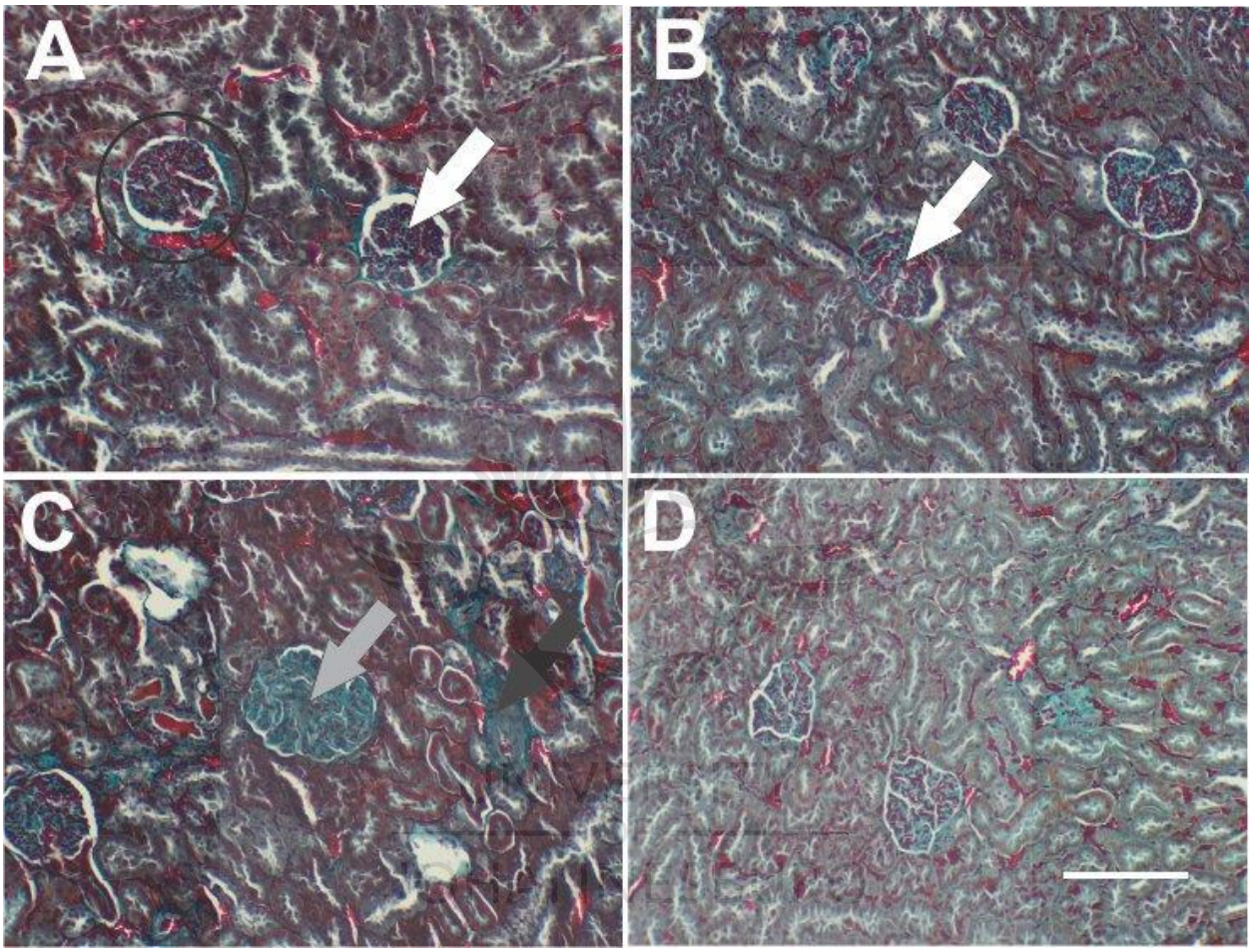

Figure 4.4: Photomicrographs showing the extent of fibrosis in the cortical region of the kidney (MT, 40X). A: Control, B: Ethanol only, C: Atripla only and D: Atripla with ethanol. White arrows (in A &B) showing the glomeruli in the renal cortex. The grey arrow in C shows extensive glomerular fibrosis in the Atripla only group. Scale Bar in D = 20µm and applies to A, B and C.

4.3.2 Renal corpuscular, glomerular tuft and urinary space areas

The renal corpuscular area was 11% and 2% higher in the animals treated with ethanol only and Atripla only respectively when compared to the control animals whereas in the Atripla with ethanol group the corpuscular area was 1% lower than the control animals (F= 3.57, P= 0.01), (Table 4.3). Similarly, the glomerular tuft area was 11% and 2% higher in the ethanol only and Atripla only groups respectively compared to the control group while in the Atripla with ethanol group, the glomerular tuft area was also 1% lower than the control group (F= 6.13, P= 0.0004), (Table 4.3). However, no statistically significant differences were observed in the urinary space area across the treatment groups (F= 1.67; P= 0.17), (Table 4.3).

4.3.3 Connective tissue area fraction in the glomerular tuft

The connective tissue area fraction in the glomerular tuft was 39%, 37% and 83% higher in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control group (F= 17.35; P= 0.0001), (Table 4.3).

Table 4.3: Renal corpuscular, glomerular tuft, urinary space areas and glomerular tuft connective tissue area fraction

	Treatment			
	Control	Ethanol	Atripla	Atripla+ Ethanol
RCA	9051.00±2211.00^{a}	10003.00±2349.00^{b}	9223.00±2495.00^{a}	8952.00±5489.00^{c}
GTA	5854.00±1789.00^{a}	6522.00±2021.00^{b}	6219.00±2032.00^{a}	5772.00±1891.00^{c}
USA	3196.00±1193.00^{a}	3464.00±1464.00^{a}	2822.00±1502.00^{a}	3180.00±5089.00^{a}
GT CT $A_{fraction}$	0.18±0.08^{a}	0.25±0.11^{b}	0.25±0.10^{c}	0.33±0.13^{d}

RCA= Renal corpuscular area, GTA= Glomerular tuft area, USA= Urinary space area, GT CT $A_{fraction=}$ Glomerular tuft connective tissue area fraction. Data of all variables expressed as mean ±standard deviation. Different superscripts indicate groups with significant differences. P <0.05

4.3.4 Proximal convoluted tubular outer, luminal and epithelial areas

On the proximal convoluted tubular outer area, no statistically significant differences were seen in the ethanol only group compared to the control group (F= 4.39; P= 0.005), (Table 4.4). However, the proximal convoluted tubular outer area was 17% and 1% lower in the Atripla only and Atripla with ethanol groups respectively compared to that of the control group (F= 4.39; P= 0.005, (Table 4.4). No statistically significant differences were observed in the proximal convoluted tubular luminal area across the treatments groups compared to the control group (F= 1.38; P= 0.25), (Table 4.4). However, the proximal convoluted tubular epithelial area was 2%, 27% and 4% lower in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control animals (F= 8.60; P= 0.0001), (Table 4.4).

4.3.5 Distal convoluted tubular outer, luminal and epithelial areas

On the distal convoluted tubular outer area no statistically significant differences were observed in the ethanol only group compared to the control group (F= 0.19; P= 0.90), (Table 4.4). However, the distal convoluted tubular luminal area was 3%, 58% and 5% higher in the ethanol only, Atripla only and Atripla with ethanol groups respectively compared to the control animals (F= 18.24; P= 0.0001), (Table 4.4). On the contrary, distal convoluted tubular epithelial area was 1%, 21% and 2% lower in the animals that received ethanol only, Atripla only and Atripla with ethanol respectively compared to the control animals (F= 6.11; P= 0.0005), (Table 4.4).

4.3.6 Interstitial Connective area fraction

The interstitial connective tissue area fraction was 83%, 88% and 113% higher in the animals that received ethanol only, Atripla only and Atripla with ethanol respectively compared to the control animals (F= 17.86; P= 0.0001), (Table 4.4).

Table 4.4: Proximal convoluted tubular, distal convoluted tubular areas and interstitial connective tissue area fraction

	Treatment			
	Control	Ethanol	**Atripla**	**Atripla+** Ethanol
PCT				
PCT OA	3407.00±1033.00[a]	3408.00±1095.00[a]	2820.00±1006.00[b]	3358.00±1263.00[c]
PCT LA	708.10±292.80[a]	774.50±380.80[a]	836.60±305.10[a]	778.20±403.60[a]
PCT EA	2699.00±831.90[a]	2634.00±820.30[b]	1983.00±945.70[c]	2580.00±982.20[d]
DCT				
DCT OA	1318.00±483.20[a]	1316.00±376.40[a]	1271.00±359.50[a]	1316.00±511.80[a]
DCT LA	296.80±159.40[a]	305.10±147.60[b]	467.90±179.40[c]	312.30±147.00[d]
DCT EA	1021.00±358.60[a]	1011.00±278.60[b]	803.00±365.10[c]	1003.00±395.80[d]
IS CT $A_{fraction}$	0.08±0.05[a]	0.15±0.05[b]	0.15±0.08[b]	0.17±0.06[b]

PCT= Proximal convoluted tubule, PCT OA= Proximal convoluted tubular outer area, PCT LA= Proximal convoluted tubular luminal area, PCT EA= Proximal convoluted tubular epithelial area. DCT= Distal convoluted tubule, DCT OA= Distal convoluted tubular outer area, DCT LA= Distal convoluted tubular luminal area, DCT EA= Distal convoluted tubular epithelial area, IS CT $A_{fraction=}$ Interstitial connective tissue area fraction. Data of all variables expressed as mean ±standard deviation. Different superscripts indicate groups with significant differences. P <0.05

CHAPTER FIVE DISCUSSION

5.0 Effects of co–administration of alcohol and Atripla on the histomorphometry of the liver and kidneys

This study investigated the effects of co–administration of alcohol and Atripla on the histomorphometry of the liver and kidneys of adult male Sprague Dawley rats. In this study the percentage weight gain of the animals treated with ethanol alone, Atripla alone or Atripla with ethanol groups was less than that of animals that did not received any treatment. These findings are in agreement with the report by others authors who reported that intake of emtricitabine and tenofovir disoproxil fumate result in weight loss (Clay *et al*., 2008; Plosker, 2013). Similarly, Borges *et al*. (2016) reported that administration of HAART medications such as atazanavir sulfate, tenofovofir disoproxil fumarate, ritonavir and lamivudine in mice resulted in lower percentage body weight gain compared to the controls. Contrary to our results, Azu *et al*. (2016) reported that oral administration of HAART medications such as stavudine, lamivudine and nevirapine resulted in higher percentage body weight gain compared to control animals. Similarly, Adewale *et al*. (2012) also recorded higher percentage body weight gain in female Wistar rats treated with a combination of lamivudine, nevirapine and zidovudine. The differences between our findings and those of (Adewale *et al*., 2012; Azu *et al*., 2016) may be due to the different regimens of medications used. We specifically noted that HAART regimens where the body weights increased did not include TDF. More so, Adaramoye *et al*. (2012) reported that the use of nevirapine did not change the body weight gain. It appears that lamivudine results in weight gain whereas TDF results in weight loss.

In the present study, the results showed a slight increase in the liver weight of the animals treated with Atripla alone or Atripla with alcohol compared to the animals that did not receive any treatment. This increase in the liver weight could be related to the increased accumulation of connective tissue in the liver observed in the current study or as a result of hepatocellular hypertrophy. These findings are consistent with the report by Adaramoye *et al*. (2012) who reported the increase in liver weight of animals treated with nevirapine. Furthermore, our findings showed a slight increase in the kidney weights of the animals treated with Atripla alone or Atripla

with alcohol compared to the ones that did not receive any treatment. Increase in the kidney weight of animals treated with ethanol alone, Atripla alone or Atripla with ethanol groups were observed in this study. This could be as a result of the excessive accumulation of collagen observed in the organs of the treated animals. The current study is the first to report on the kidney and liver weight changes after administration of Atripla and alcohol, therefore no comparisons could be drawn in this regard.

5.1 Effect of the treatments in the Liver

The findings from this study revealed a reduction in the size of the hepatocytes in zone I of the liver in animals treated with ethanol alone, Atripla alone or Atripla with alcohol compared to the control group. The decreased size of the hepatocytes in zone I could be suggestive of hepatocellular atrophy. This could be as a result of the shrinkage secondary to the toxic effect of the treatments. However, increases in size of the hepatocellular nuclei in zone I were observed in animals treated with Atripla alone or Atripla with alcohol. This increase in nuclear sizes could be related to resumption of mitotic cell division. Increased mitotic activity might occur due to xenobiotic-induced lesions resulting into regenerative nodular hyperplasia (Thoolen *et al*., 2010). Xenobiotics induce an increase in microsomal enzymes and organelles leading to hepatocellular hypertrophy (Thoolen *et al*., 2010).

In zone 3 there was an increase in the size of the hepatocytes which could be as result of the cells growing bigger and rearranging its organelles in preparation for mitotic division (Clemens, 2007). Increases in size of hepatocytes might suggest hepatocellular hypertrophy that is usually observed during liver regeneration (Michalopoulos and DeFrances, 2005; Michalopoulos, 2007; Alison *et al*., 2009). These findings are similar to those reported by Fazelipour *et al*. (2008) who reported heroin induced increases in the size of Zone 3 hepatocytes. Vento and Cainelli (2002) also reported an increase in the size of zone 3 hepatocytes in alcohol treated animals. In addition, hepatocellular hypertrophy observed in zone 3 hepatocytes of animals treated with ethanol alone, Atripla alone or Atripla with alcohol might be xenobiotic induced since the hepatocytes in zone 3 are responsible for glycolysis, lipogenesis, and cytochrome P450 based drug detoxification (Thoolen *et al*., 2010).

Furthermore, increases in size of the hepatocyte nucleus in zone 3 were observed in animals treated with ethanol alone, Atripla alone or Atripla with alcohol. Increases in size of the hepatocytes nucleus is presumably related to xenobiotic induced over activity of hepatocytes or necrotic injury caused by ethanol alone, Atripla alone or Atripla with alcohol (Thoolen *et al*., 2010). In addition, the increases in size of the hepatocellular cytoplasm observed in zone 3 of animals treated with Atripla alone or Atripla with alcohol compared to control group could be as a result of vacuolization of the cytoplasm observed in this study (Fazelipour *et al*., 2008).

The increase in connective tissue of the liver recorded in the current study in the animals treated with Atripla alone or Atripla with alcohol might suggest fibrosis secondary to toxicity of the drugs. Fibrosis occurs secondary to inflammation in the liver due to liver injury caused by the treatments. At the site of inflammation, hepatic stellate cells become activated to complement myofibroblast thereby leading to variation in collagen composition (Xu *et al*., 2012).

5.2 Effect of the treatments in the kidneys

In the kidney, the increase in size of the renal corpuscle in ethanol alone and Atripla alone treated groups, may suggest Bowman's capsule expansion due to glomerular hyper filtration. According to Kotyk *et al*. (2016), glomerular hyper filtration causes Bowman's capsule expansion as a result of developed glycation and absence of the tetraspan in CD151. In addition, the increase in size of the glomerular tuft in animals treated with ethanol alone and Atripla alone may suggest inflammation due to podocytes hypertrophy and the abnormal mesangial expansion as a result of glomerular hyper cellularity. Mesangial expansion happen as a result of high deposition of extracellular matrix proteins such as fibronectin into the mesangium (Mason and Wahab, 2003). Although, the Bowman's capsule and the glomerular tuft sizes increased, the urinary space was not affected. Furthermore, the increase in size of the glomerular tuft connective tissue observed in ethanol alone or Atripla alone groups maybe due to fibrosis resulting from the accumulation of the connective tissue in the glomerular tuft as a result of glomerulosclerosis (Alsaad and Herzenberg, 2007). Alsaad and Herzenberg (2007) stated that glomerulosclerosis occur by the accumulation of proteins in the mesangial cell resulting in fibrosis. However, Mason and Wahab (2003) reported that the extracellular matrix proteins are collagen and fibronectin. Accumulation of these two

occur due to expansion of the mesangial cells and matrix metalloproteinases atrophy (Chen *et al*., 2003).

The decreases in size of the proximal convoluted tubule in Atripla alone or Atripla with alcohol group may suggest proximal tubulopathy (Labarga *et al*., 2009). Proximal tubulopathy may occur due to nephrotoxicity of TDF on tubular cells as a result of acute tubular necrosis (Herlitz *et al*., 2010). In addition, the luminal size increase of proximal tubule of animals treated with ethanol alone, Atripla alone or Atripla with alcohol may be due to the concomitant toxic effects of the treatment. The increase in luminal size can be explained by the decrease in size of the epithelial cells in the walls of the proximal convoluted tubule. These findings are in agreement with those of Ramamoorthy *et al*. (2017) who recorded that the proximal convoluted tubules of adult male Wistar rats treated with TDF were disfigured, the lining of the epithelial cell were obliterated, and interstitial fluid and vacuolization of the cytoplasm were present. Similarly, Quinn *et al*. (2010) reported the morphological changes in proximal tubule, the study indicate that the principal location of toxicity in patient treated with TDF is the proximal tubule. Damages to the proximal tubular cells in patient using TDF may likely result in renal failure (Izzedine *et al*., 2005c; Kohler *et al*., 2009).

Regarding the distal convoluted tubule, the reduction in size of the epithelial area coupled with increase in the lumen size in Atripla or Atripla with alcohol may suggest drug induced tubular injury of the distal tubules as a result of concomitant toxic effect of the treatments.

Finally, the increased accumulation of the interstitial connective tissue area in the treatment groups could be secondary to the toxicity of the drugs. These findings are in agreement with (Rossert, 2001; Izzedine *et al*., 2005b) who reported that drugs such as TDF resulted in tubulointerstitial injury which can lead to acute tubular necrosis and interstitial fibrosis. Tubulointerstitial fibrosis is identified as a continuous deleterious connective tissue deposition on the kidney tubular cell which appears as a harmful process leading to renal degeneration (Efstratiadis *et al*., 2009).

5.3 Limitation of the study

This study did not measure cell proliferation, measurement of the cell proliferation would have given us the amount of number of cells that is dividing or change in the proportion of cells. Additionally, drug-drug interactions are not performed in this study, it would have enabled our findings to figure out which of the components of Atripla with concomitant alcohol interacts together to form increased or decreased formation of toxic metabolites. Also, immunohistochemistry test were not performed, this would have assess specific tumor antigens or inflammatory factors.

5.4 Recommendation

Although Atripla is said to reduced morbidity and mortality, however, findings from this study have revealed that co-administration of Atripla and ethanol result in liver injury and renal damage such as centrilobar hepatocellular hypertrophy, sinusoidal dilation, intestistial fibrosis and tubular dilatation. Therefore, clinicians should give advice to their patients regarding this medication and the consequences thereafter when administering it with concomitant alcohol abuse. For future studies measurement of the cell proliferation should be put into consideration. Drug-drug interactions should be performed on the components of Atripla. Immunnohistochemistry test should be performed so as to know the types of inflammatory cells present.

5.5 Conclusions

The effects of Atripla with co–administration of alcohol was determined on the liver and kidneys. Findings from this study revealed that the co–administration of atripla and ethanol is associated with less body weight gain. Furthermore, our findings has shown that the use of Atripla alone or with alcohol in the liver are associated with hepatotoxicity in vitro. As seen from the results increase in liver weight may be associated with hepatocellular hypertrophy. Furthermore, the use of Atripla or with alcohol has proved to have drastic effects on the hepatic cells as a result of hepatocellular atrophy in zone 1 and centrilobar hepatocellular hypertrophy in zone 3. In addition,

adverse effects of these treatments such as inflammation, fibrosis and sinusoidal dilatation has been observed.

Similarly, the use of Atripla alone or with co–administration of alcohol in the kidneys are associated with nephrotoxicity in vitro. Increased in the kidney weight of the rats were also observed as a result of toxic effects of the treatments. Our findings have shown damages caused as a result of deleterious concomitant effect of this treatments on the renal corpuscles and renal tubules such as renal corpuscular and glomerular hypertrophy, tubular epithelial atrophy accompanied by tubular dilatation and interstitial fibrosis.

Therefore concomitant use of Atripla alone or with alcohol have shown to be associated with liver injury, renal damage and inflammation.

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APPENDICES

APPENDIX A

HDC CLEARANCE LETTER

UNIVERSITY OF JOHANNESBURG

FACULTY OF HEALTH SCIENCES

HIGHER DEGREES COMMITTEE

HDC-01-46 - 2017

11 July 2017

TO WHOM IT MAY CONCERN:

STUDENT: DARAMOLA, OF
STUDENT NUMBER: 201422762

TITLE OF RESEARCH PROJECT: The Effects of Atripla and Alcohol Co-Administration on the Histomorphometry of the Liver and Kidneys of Adult Male Sprague Dawley Rats

DEPARTMENT OR PROGRAMME: BIOMEDICAL TECHNOLOGY

SUPERVISOR: Mr P Nkomozepi CO-SUPERVISOR: Mr N Xhakaza

The Faculty Higher Degrees Committee has scrutinised your research proposal and concluded that it complies with the approved research standards of the Faculty of Health Sciences; University of Johannesburg.

The HDC would like to extend their best wishes to you with your postgraduate studies

Yours sincerely,

Prof BS Shaw
Chair: Faculty of Health Sciences HDC
Tel: 011 559 6891
Email: brandons@uj.ac.za

APPENDIX B

ETHICAL CLEARANCES

1. **M&E FROM WITS**
2. **ETHICS CLEARANCE FROM UJ**

AESC 2012 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Trust Nyirenda

b. School and email address: School of Anatomical Sciences; trust.nyirendah@gmail.com

c. Experiment to be modified / extended

		AESC NO	
Original AESC number	2015	08/33	C
Other M&Es : Request for addition of Co-workers to the study Request for addition of Co-workers to the study and capturing images Request for Rat Carcasses to refine methodology techniques			3

d. Project Title: Effects of Atripla® and/or alcohol with protein malnutrition on the testis, epididymis and prostate glands of adult male Sprague Dawley rats.

		No.	Species
e.	Number and species of animals originally approved:	80	*Sprague Dawley*
f.	Number of additional animals previously allocated on M&Es:	nil	
g.	Total number of animals allocated to the experiment to date:	78	
h.	Number of animals used to date:	2	

i. Specific modification / extension requested:

Request for permission to add Ms Olukemi Funmilayo Daramola, student number 201422762 MTech University of Johannesburg, as a co-worker in the study.

j. Motivation for modification / extension:

Ms Daraloma will provide technical assistance which includes liver and kidney histology after termination of the experimental animals.

Date: 25/05/2017

Signature:

RECOMMENDATIONS: Approved. Inclusion of Ms Daraloma as a co-worker to provide technical assistance with histology.

Date: 29 May 2017

Signature:

Chairman, AESC

UNIVERSITY OF JOHANNESBURG

FACULTY OF HEALTH SCIENCES

RESEARCH ETHICS COMMITTEE

NHREC Registration no: REC-241112-035

REC-01-70- 2017

11 July 2017

TO WHOM IT MAY CONCERN:

STUDENT: DARAMOLA, OF
STUDENT NUMBER: 201422762

TITLE OF RESEARCH PROJECT: The Effects of Atripla and Alcohol Co-Administration on the Histomorphometry of the Liver and Kidneys of Adult Male Sprague Dawley Rats

DEPARTMENT OR PROGRAMME: BIOMEDICAL TECHNOLOGY

SUPERVISOR: Mr P Nkomozepi **CO-SUPERVISOR:** Mr N Xhakaza

The Faculty Academic Ethics Committee has scrutinised your research proposal and confirm that it complies with the approved ethical standards of the Faculty of Health Sciences; University of Johannesburg.

The REC would like to extend their best wishes to you with your postgraduate studies.

Yours sincerely,

Dr C Stein
Chair : Faculty of Health Sciences REC
Tel: 011 559 6564
Email: cstein@uj.ac.za

APPENDIX C

TURNITIN CERTIFICATE

ATRIPLA

ORIGINALITY REPORT

23%	17%	16%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Musabayane, C.T.. "Effects of ethanol on the changes in renal fluid and electrolyte handling and kidney morphology induced by long-term chloroquine administration to rats", Alcohol, 200011 Publication	<1%
2	cid.oxfordjournals.org Internet Source	<1%
3	Th. Christofides. "Elements of the Biliary Tract and Liver Physiology", Liver and Biliary Tract Surgery, 2006 Publication	<1%
4	Rusyn, Ivan, and Ramon Bataller. "Alcohol and toxicity", Journal of Hepatology, 2013. Publication	<1%
5	Submitted to University of Witwatersrand Student Paper	<1%
6	www.hindawi.com Internet Source	<1%

7	Submitted to Mansoura University Student Paper	<1%
8	aidsinfo.nih.gov Internet Source	<1%
9	www.comed.uobaghdad.edu.iq Internet Source	<1%
10	link.springer.com Internet Source	<1%
11	"PanVascular Medicine", Springer Nature, 2015 Publication	<1%
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