NEPHROPROTECTIVE AND HEPATOPROTECTIVE EFFECTS OF *TERMINALIA IVORENSIS* A. CHEV. ETHANOLIC STEM BARK EXTRACT ON SPRAGUE DAWLEY RATS

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By

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmacology, KNUST. This work has not been presented for any other degree.

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Rev. Prof. Charles Ansah Date

(Supervisor)

DEDICATION

I dedicate this work to my family.

ABSTRACT

Terminalia ivorensis has been West African ethnomedicine for the management of several diseases. For instance, to treat ulcers, wounds, general body pains, hemorrhoids, diuresis, malaria and yellow fever. In this study, *T. ivorensis* was assessed for potential nephroprotective and hepatoprotective actions using potassium dichromate and gentamycin as nephrotoxicant and hepatotoxicant respectively in Sprague Dawley rats.

The extract (100 – 5000 mg/kg) administered to the rats for 24 hours in an acute toxicity study did not show undesirable effects on the general behavior of rats and no death was recorded. The LD_{50} was estimated to be above 5000 mg/kg of extract.

In the nephroprotective study, administration of potassium dichromate (20 mg/kg; sc) caused elevation of creatinine from the control value of 27.83 μmL/L to 358.70 μmL/L. Urea levels also increased from 5.09 mmol/L to 56.55 mmol/L. Administration of the *T. ivorensis* extract with the nephrotoxicant reduced the elevated creatinine to 123.70 μmL/L and urea to 27.81 mmol/L at the highest dose of 1000 mg/kg of the extract. The reduced levels of endogenous antioxidants observed with potassium dichromate treatment, was also significantly reversed by *T. ivorensis* extract. Similar results were obtained with gentamycin as the nephrotoxicant.

In the hepatoprotective investigations using gentamycin as hepatotoxicant, *T. ivorensis* the elevated levels of AST, ALT and GGT from 985.5 U/L to 361.2 U/L, 218.8 U/L to 126.2 U/L and 4.6 U/L to 2.0 U/L respectively. The Histopathological studies confirmed that *T. ivorensis* extract is nephroprotective and hepatoprotective in rats by a mechanism possibly related to the antioxidant effect of the extract.

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LIST OF ABBREVIATIONS

ALP – Alkaline phosphatase

ALT – Alanine aminotransferase

AST – Aspartate aminotransferase

ANOVA – Analysis of variance

BUN – Blood urea nitrogen

DTNB – 5,5‟-dithiobis-2-nitrobenzoic acid

Gent – Gentamycin

GGT – Gamma – glutamyl transferase

GPx – Glutathione peroxidase

GSH – Reduced glutathione

GSSG – Glutathione disulfide

GST – Glutathione-S-transferase

HOCl – Hypochlorous acid

$K_2Cr_2O_7$ – Potassium dichromate

LD50 – Median lethal dose

MDA – Malondialdehyde

MPO – Myeloperoxidase

NADH – Nicotinamide adenine dinucleotide

NBT – Nitro blue tetrazolium

PMS – Post mitochondrial supernatant

PUFA – Polyunsaturated fatty acids

RBCs – red blood cells

RNS – Reactive nitrogen species

ROS – Reactive oxygen species

SD – Sprague Dawley

SEM – Standard error of mean

SGOT – Serum glutamic oxaloacetic transaminase

SGPT – Serum glutamate pyruvate transaminase

TIE – *Terminalia ivorensis* extract

CHAPTER ONE INTRODUCTION

1.1 OVERVIEW

Nephrotoxicity is a disease of the kidneys caused a drug and /or other agents. Available record shows that 27 million persons have chronic kidney disease, indicating nearly one in every seven adults and a 30 % rise over the past decade (Coresh *et al*., 2007). More than 200,000 people in the US suffer from kidney failure (USRDS, 2008). The frequency of non-terminal kidney failure in Swedish children 1 to 15 years was 4.50 per million total population (Pratibha *et al.,* 2009). Renal inefficiency is seen in nearly 2 % of patients with paracetamol overdose and the occurrence of renal dysfunction from aminoglycosides increased from 3 % in 1969 to 10 – 20 % in 2010 (Rajitha *et al.*, 2013; Eslami, *et al.*, 2011).

Thus, nephrotoxicity remains a challenging side effect of various classes of drugs including cyclosporine, aminoglycosides, vancomycin and amphotericin B, as well as diagnostic agents like radio contrast media, and this limits their medical uses (Rajitha *et al.*, 2013). Renal injury is also caused by various compounds such as potassium dichromate used in various industries (e.g. metallurgy, chrome plating, paints, leather tanning, photography and photoengraving, refractory and stainless steel, metal finishes, textile manufacture, wood preservation and cooling systems) (Rajitha *et al.*, 2013; Pedraza - Chaverri, *et al.*, 2008).

Another common and relevant adverse effect of drug therapy is hepatotoxicity. It is a global problem since it accounts for 5 % of all hospitalized cases and 75 % of adverse drug reactions resulting in death (Sivakrishnan and Kottaimuthu, 2013; Aashish *et al.*, 2012). It

is also a common cause of drug withdrawal from the market (Hartmut *et al.*, 2001; Rolf, 2009).

Medicinal plants are used to treat various human diseases and are getting more consideration as a result of their less toxicity and great efficacy (Aslam *et al.*, 2013). Potential health benefits of these plants have triggered a rising interest in their analysis and research. As a result, data on scientific screening of plant extracts (both crude and purified) is accumulating gradually but steadily (Bulus *et al.*, 2011). Works on toxicity, antihyperglycemic, anti-malarial and nutraceutical properties of plant – based products support this claim (Johnny *et al.*, 2014; Adinortey *et al.*, 2012; Annan *et al.*, 2012; Ansah *et al.*, 2009). Among the medicinal plants used for the treatment of various diseases is *Terminalia ivorensis*.

Terminalia ivorensis is a tree located in the tropical and sub-tropical zones (Orwa *et al.*, 2009; Burkill, 1985). The plant is known in French as framire, Spanish as terminalia, English as black afara and Swahilli as mwalambe (Orwa *et al.*, 2009). Its native names are bassi (Guinea-Bisau); kahuri (Ivory Coast); bai-ti (Liberia); badi (Sierra Leone); awunshin (Igbo, Nigeria); afara dudu (Yoruba, Nigeria); egboen-nebi (Edo, Nigeria) (Odeyemi, 2010; Burkill, 1985). In Ghana, *T. ivorensis* is known as amire in Ashanti, Fanti, Wassa and Ahanta whilst Ga and Krobo call it emere. In Nzema, Brosa, Esa and Anyi, it is termed frammire and as dzogbedodo amongst the Ewes (Safra, 2003; Burkill, 1985).

The genus *Terminalia* derives its Latin name (terminalia = end) from the location of the leaves, which are crowded at the ends of the shoots (Safra, 2003; Burkill, 1985). Several traditional uses have been associated with this plant. For instance, to treat ulcers, cuts, sores, wounds, general body pains, hemorrhoids, diuresis, malaria and yellow fever (Ouattara *et*

al., 2013; Akinyemi *et al.*, 2006; Etukudo, 2003; Burkill, 1985). It also serves as a source of timber for the construction industries (Safra, 2003; Burkill, 1985; Smith, 1971). The wood is again used for charcoal and firewood (Orwa *et al.*, 2009).

The present study focused on assessing the possible nephroprotective and hepatoprotective effects of *Terminalia ivorensis* stem bark extract against potassium dichromate and gentamycin induced nephrotoxicity and hepatotoxicity and predict the possible mechanism of protection using animal models.

1.2 THE PLANT: *TERMINALIA IVORENSIS*

Terminalia ivorensis commonly known as black afara is a member of the family Combretaceae (Orwa *et al.*, 2009; Hawthorne, 1995) and it is located in tropical and subtropical zones (Orwa *et al.*, 2009).

It is a large forest tree of height 15 – 50 m and branchless for up to 30 m. The mature plant has a leveled top with widespread horizontal canopy of uniformly dispersed flora rising from the tip of a straight stem. For the young plant, the branches are whorled, young flora fall off after a period of development (Orwa *et al.*, 2009; Safra, 2003; Adewunmi *et al.*, 2001).

The leaves of *T. ivorensis* are simple, oval – shaped and blunted tip with orange – brown hairs. Its flowers are bisexual and the fruits are winged, woody, reddish- brown in color and are variable in size. It is supported by tap root and lateral roots (Orwa *et al.*, 2009; Safra, 2003), and the bark is often blackish with deep longitudinal fissures (Orwa *et al.*, 2009).

The tree can be propagated sexually by the use of viable seeds or vegetatively by the use of cuttings and stumps (Orwa *et al.,* 2009; Burkill, 1985).

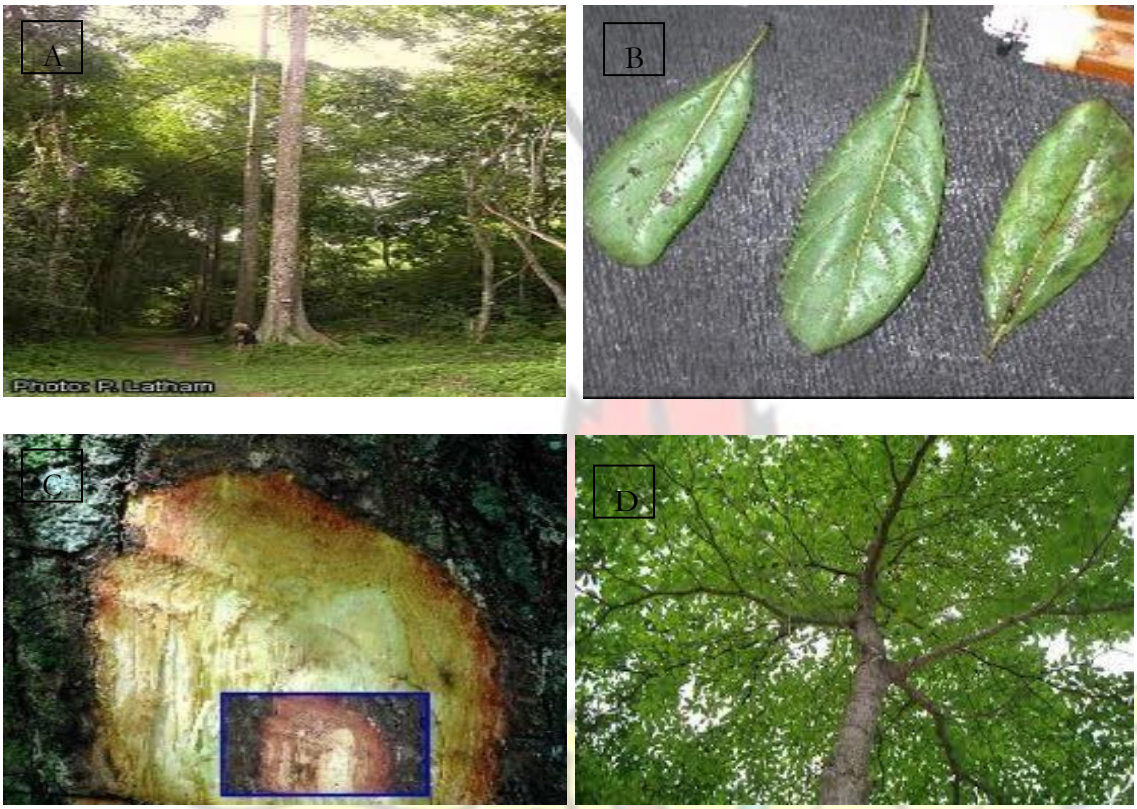


Figure 1.1: Pictures of *T. ivorensis*; A. stem, B. Leaves, C. Stem with bark removed and D. Stem showing leaves and branches.

Source: Adapted from www.ox.ac.uk and www.gstatic.com.

1.2.1 Taxonomy

Terminalia ivorensis is classified as follows:

Domain: Eukaryota

Kingdom: Plantae

Sub kingdom: Tracheobionta

Phylum: Angiosperm

Class: Eudicots

Order: Myrtales

Family: Combretaceae

Genus: Terminalia

Species: Ivorensis

Specific epithet: Ivorensis (combretaceae) A. Chev.

Botanical name: *Terminalia ivorensis* A. Chev.

1.2.2 Geographical distribution

The family Combretaceae is dispersed in the tropical and sub-tropical zones (Orwa *et al.*, 2009). *Amire*, as the plant is commonly known in Ghana, is mostly located in Africa between Guinea and Cameroon (Safra, 2003; Burkill, 1985). It is also found in Fiji, Zambia, Tanzania, China, Uganda, South Africa, Trinidad and Tobago, Solomon Islands and Zimbabwe (Orwa *et al.*, 2009).

1.2.3 Traditional Uses

Terminalia species provide economic, medicinal, social and spiritual benefits. The trees are in several countries in the tropics as a source of high quality solid timber for carving, carpentry, building and construction (Safra, 2003; Burkill, 1985; Smith, 1971) and the wood is also used for charcoal and firewood (Orwa *et al.*, 2009).

Traditional faith healers use the bark decoctions of *T. ivorensis* to treat diarrhoea, diabetes, hypertension, parasitic diseases and coughs as well as cutaneous, buccal and teeth infections (Sitapha *et al.*, 2013). It is also used for the treatment of ulcers, cuts, wounds, general body pains, hemorrhoids, diuresis, malaria and yellow fever (Ouattara *et al.*, 2013; Akinyemi *et al.*, 2006; Etukudo, 2003; Burkill, 1985). The sap from the leaves is used as aphrodisiac and for the treatment of venereal diseases and kidney infections (Ouattara *et al.*, 2013; Burkill, 1985). Extracts of the bark which yield yellowish-red pigment are used as dyes, inks, stains, tattoos and mordants (Iwu and Anyanwu, 1982; Burkill, 1985).

In Africa, forests are used for spiritual purposes. Trees of *T. ivorensis* are believed to link the living with their ancestors, as this is often symbolized in the relationship between the earth and the sky. Socially, the trees offer good shade under which banana, coffee and cocoa are planted (Orwa *et al.*, 2009).

1.2.4 Previous works on *T. ivorensis*

The bark decoctions of *Terminalia ivorensis* enjoyed a wide array of medicinal and phytochemical assessments. The phytochemical analysis of the stem bark of *T. ivorensis* showed the presence of tannins, saponins, flavonoids, alkaloid, terpenes, steroids, coumarins, anthraquinones, cardiac glycosides and polyphenols (Coulibaly *et al.*, 2014; Johnny *et al.*, 2014; Annan *et al.*, 2012).

Ouattara *et al.*, (2013) reported the antifungal activities of *Terminalia ivorensis* A. Chev bark extracts against *Candida albicans* and *Aspergillus fumigatus* whilst Coulibaly *et al.*,

(2014) reported the antibacterial properties of the trunk bark of *T. ivorensis* against methicillin-resistant Staphylococci spp strains. In other works, Johnny *et al.,* (2014), Annan *et al.,* (2012) and Agbedahunsi *et al.,* (2006) respectively reported the anti-hyperglycemic, anti-malarial and trypanocidal properties of the bark extract of *T. ivorensis*. Other biological activities associated with the plant include anti-arthritis and anti-inflammatory properties (Iwu and Anyanwu, 1982).

1.3 THE KIDNEY

1.3.1 Structure of the kidney

The kidneys are bean – shaped and reddish brown in color (Van De Graaff, 2001), found in the abdominal cavity and located on both sides of the vertebral column (Saladin, 2003). They lie beside the posterior abdominal wall at the level of the twelfth thoracic and third lumbar vertebrae (Van De Graaff, 2001). The two kidneys are surrounded and shielded by the lower ribs and the lower portion of the diaphragm rests on their upper parts (Saladin, 2003).

The liver occupies a large space on the right side, thus, displacing the right kidney about 1.5 cm lower than the left one (Van De Graaff, 2001). The adult human kidney weighs about 160 g, and measures about 2.5 cm thick, 11.25 cm long and 5.5 – 7.7 cm wide (Saladin, 2003; Van De Graaff, 2001).

The kidney is sheltered by 3 layers of tissue. These layers include: (1) the renal capsule, an innermost strong and transparent fibrous layer which is attached to the hilum and shields the kidneys from shock and infection, (2) the adipose capsule, a fatty tissue which pads the

kidney and grips it in position and (3) the renal fascia, a fibrous connective tissue which clamps the kidney and related organs to the abdominal wall (Scanlon and Sanders, 2007; Saladin, 2003; Van De Graaff, 2001).

Each kidney has lateral convex and medial concave surfaces with a depression called the hilum on its mid surface. Through the hilum, there is entry of renal artery and nerves as well as exit of the renal vein and ureter. Also, drainage of lymphatic vessels take place at the hilum (Scanlon and Sanders, 2007; Saladin, 2003; Van De Graaff, 2001). The abdominal aorta subdivides into the renal artery; blood is returned to the inferior vena cava through the renal vein and urine is conveyed from the kidneys through the ureter to the bladder (Scanlon and Sanders, 2007).

Gross examination of the coronal or frontal segment of the kidney exposes two anatomically distinct regions: the outer renal cortex and the renal medulla (Saladin, 2003). The outer renal cortex, about 1 cm thick (Saladin, 2003), is reddish brown and granular in appearance due to the existence of many capillaries (Van De Graaff, 2001) and it consists of renal corpuscles and convoluted tubules (Scanlon and Sanders, 2007). It is the main part of the kidney and gets a disproportionately greater percentage (90 %) of blood flow in relation to the medulla (6 – 10 %) or 1 – 2 % to papilla (Klaassen, 2008). Thus, a higher percentage of a blood – borne toxicant received by the kidney is transported to the cortex, consequently, having higher chance to affect cortical than medullary or papillary functions (Davies *et al*., 2008; Klaassen, 2008). However, prolong exposure of toxicant reaches luminal concentrations that affect medullary and papillary tissues (Klaassen, 2008).

The renal medulla however is dark and striped in appearance due to the presence of microscopic tubules and blood vessels (Van De Graaff, 2001). It is composed of loops of

Henle and collecting ducts (Scanlon and Sanders, 2007). Projections of the renal cortex called renal columns split the medulla into 6 – 10 renal pyramids (Saladin, 2003) and the apexes of the pyramids are called renal papillae. The papilla extends into a small indentation called minor calyx, which collects urine. The major calyces link to form a funnel – shaped renal pelvis for collecting urine and carrying it into the ureter (Van De Graaff, 2001). The ureter is a tubular prolongation of the renal pelvis, which conveys urine into the urinary bladder (Saladin, 2003).

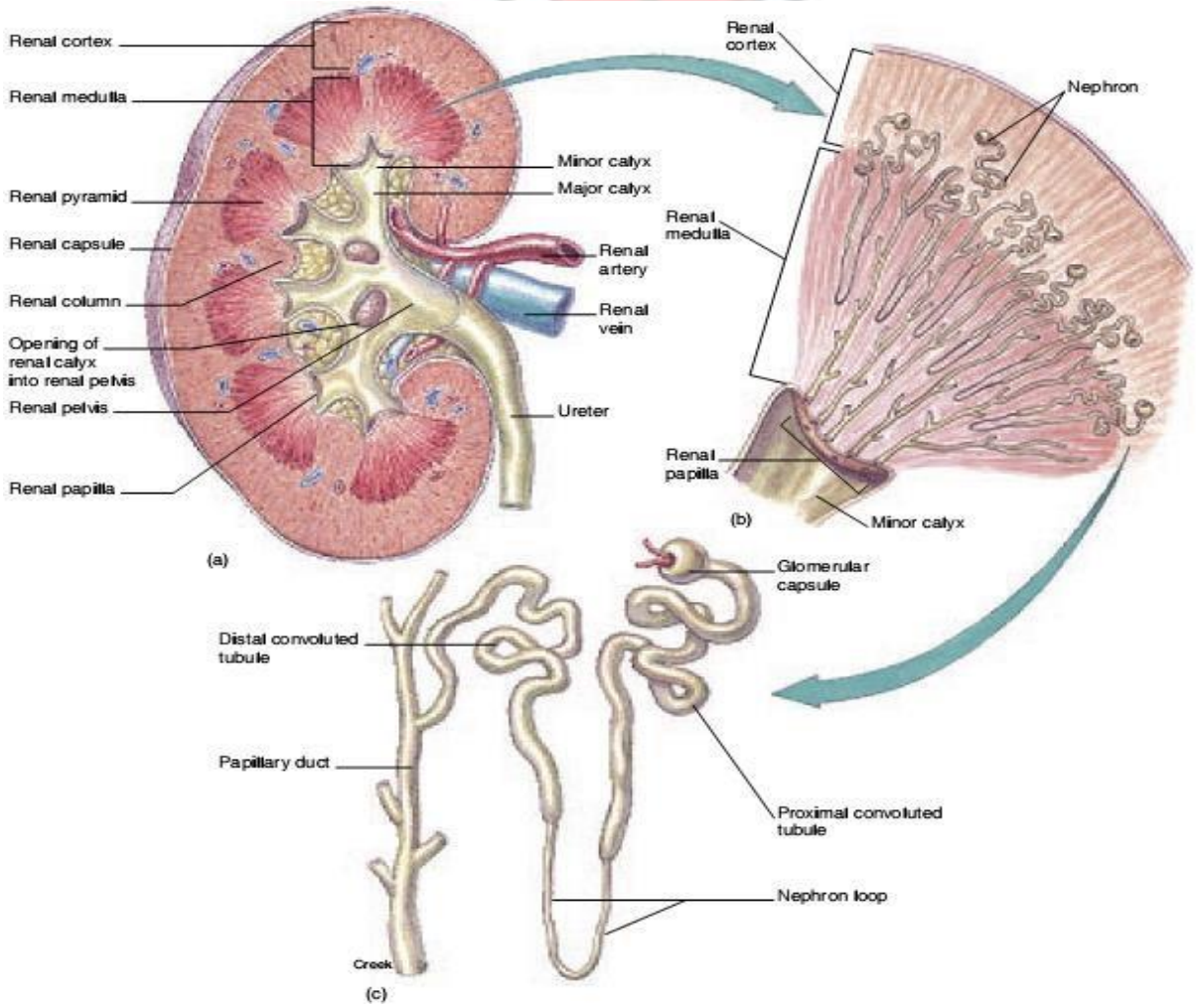


Figure 1.2: The internal structures of a kidney. (a) A coronal section showing the renal cortex, renal medulla and renal pelvis. (b) A diagrammatic magnification of a renal pyramid to depict the renal tubules. (c) A diagram of a single nephron and a papillary duct.

Source: Adapted from Van De Graaff (2001).

1.3.2 Functions of the kidney

The kidney functions principally as an organ for excretion of metabolic waste and maintenance of blood pH, fluid volume and electrolyte composition (Klaassen, 2008; Scanlon and Sanders, 2007). The processes involved in the removal of waste and maintenance of fluid composition and volume leads to the formation of urine (Klaassen, 2008).

The fundamental unit of the kidney involved in these processes is known as the nephron (Scanlon and Sanders, 2007). The tubular nephron consists of glomerular capsule, proximal convoluted tubule, descending loop of Henle, ascending loop of Henle and distal convoluted tubule (Van De Graaff, 2001).

In forming urine, the kidneys change plasma into urine through filtration, reabsorption and secretion (Scanlon and Sanders, 2007; Saladin, 2003). Blood from circulation enters the glomerulus where it is filtered and the filtrate moves into the Bowman‟s capsule. Glomerular filtration is done only in terms of size, thus, large molecules stay in the blood. Only 1 % of the filtrate is converted into urine but 99 % of it is reabsorbed into the blood (Scanlon and Sanders, 2007; Van De Graaff, 2001).

As the filtrate moves from the Bowman‟s capsule to the proximal convoluted tubule through to the distal convoluted tubule, salts and other molecules are reabsorbed through active transport, whereas water is reabsorbed by osmosis, negative ions by passive diffusion

and small proteins through pinocytosis are taken up by proximal tubule cells (Scanlon and Sanders, 2007; Saladin, 2003). Tubular secretion occurs from the blood into the filtrate in the renal tubule. Creatinine, ammonia and other waste products are secreted into the filtrate, thus, changes the composition of the tubular fluid whereas hydrogen ions are secreted to help maintain blood pH (Scanlon and Sanders, 2007; Saladin, 2003; Van De Graaff, 2001).

As the tubular fluid leaves the distal convoluted tubule into the collecting duct, it is called urine, which is then stored in the urinary bladder and later excreted (Saladin, 2003). Other homeostatic roles of the kidney include: detoxifying poisons, activation of vitamin D, synthesis of glucose, production of erythropoietin, secretion of renin, controlling blood pressure and pressure of oxygen and carbon dioxide of the blood (Scanlon and Sanders, 2007; Saladin, 2003).

1.3.3 Toxicity to the kidney

The vulnerability of the kidney to damage by a toxicant depends on the kidney‟s blood supply, position in the body, function and the presence of biochemical pathway. For instance, the kidney occupies 0.5% of total body weight, yet receives 20 – 25% of cardiac output, subsequently exposing it to relatively high amounts of toxicants in the blood. The mechanisms involved in producing urine too aid to concentrate possible toxicants in the tubular fluid. Consequently, a non – toxic amount of a substance in the blood may get to toxic levels in the kidney. Thus, renal metabolism, accumulation and distribution of substances considerably make it prone to injury by toxins (Klaassen, 2008).

Nephrotoxicity (Greek: nephros, “kidney”) is the term used for the toxic effect of some substances, (both chemicals and medications), on the kidney. Nephrotoxins are substances exhibiting nephrotoxicity (Vidya *et al.*, 2013; Galley, 2000). There are various types of toxicity: drug – induced, infection related and immune complex – related.

Drug-induced nephropathy is presented by reduced urine concentrating ability, proteinuria, glucosuria, lysosomal enzymuria, diminished excretion of ammonium and decreased rate of glomerular filtration. Drug – induced toxicity is now considered to be allergic in nature, resulting in immune – mediated injury (Vidya *et al.*, 2013).

1.3.3.1 Pathogenic Mechanisms of toxicity

Numerous mechanisms lead to renal dysfunction following exposure to chemical substances. Most substances shown to induce nephropathy exert their effects through one or more pathogenic means (Portilla *et al*., 2013). Some cause renal dysfunction through direct effect to the renal tubules and accumulation of the substance in the renal tubule (Rajitha *et al.*, 2013). The mechanism of nephrotoxicity is unclear in most occasions. Vulnerability of the kidney to toxins is usually linked to the complexities of its anatomy and physiology (Maryann and Jeanmarie, 2008). For instance, blood flow in the kidney is unequally distributed, with its cortex getting a disproportionately higher amount compared to medulla and papilla (Klaassen, 2008). Consequently, blood borne toxins are conveyed more to the renal cortex and so having a higher likelihood to affect cortical than medullary or papillary functions (Davies *et al*., 2008; Klaassen, 2008).

The mechanisms of forming urine also serve to accumulate potential toxicants existing in the filtrate (Davies *et al*., 2008; Scanlon and Sanders, 2007; Saladin, 2003; Van De Graaff, 2001). Especially, reabsorption in the nephron may elevate the tubular concentration of a toxic substance in the proximal tubule (10 – 50 mM), loop of Henle (66 mM), the distal tubule (200 mM) and 2000 mM in the collecting duct (Davies *et al*., 2008). Further concentration of toxin may lead to precipitation of poorly soluble substances, resulting in acute renal failure (Davies *et al*., 2008; Scanlon and Sanders, 2007).

Again, proximal tubular epithelium is more permeable than the distal tubular epithelium, as a result, there is preferential delivery of toxic substances to the proximal tubular epithelium and hence, injuring it (Davies *et al*., 2008). Active transport occurring in the proximal tubule further increases the intracellular concentration of a toxicant (Davies *et al*., 2008; Scanlon and Sanders, 2007). Some portions of the nephron also metabolically bioactivate protoxicants to active toxicants (Davies *et al*., 2008; Van De Graaff, 2001).

1.3.4 Assessment of kidney function

Periodic examination of kidney function is a part of medical care for adults and chronically sick individuals (Stevens *et al.*, 2006). The drug manufacturers, regulatory institutions and healthcare providers are the major stakeholders in the improvement of markers for assessing kidney function. The innovation of organ-specific parameters for assessing kidney function gives each stakeholder a better drug development opportunity that ultimately give rise to safer and more effective products delivery (Rouse *et al.*, 2012).

Blood urea nitrogen and creatinine are the commonly used biomarkers for the assessment of kidney injury. These are however not specific to the cause or location of the injury, hence, there is a great need for biomarker improvement (Goodsaid *et al.,* 2009). „„Omic"" technologies (e. g. genomics and proteomics) may provide a great potential to realizing this need (Shao *et al.*, 2011; Ling *et al.*, 2010; Fliser *et al.*, 2009).

1.3.4.1 Blood Urea Nitrogen (BUN)

Urea is the principal waste product of protein catabolism. It is produced in the liver from ammonia which is formed as a result of the deamination of amino acids. In the urea cycle, N-acetylglutamate regulates the production of urea through its allosteric activation of carbamoyl phosphate synthetase I, the enzyme in the rate limiting step of the pathway (Nelson and Cox, 2005). Urea is dissolved in the blood and is mainly excreted by the renal tubules and a small amount in the sweat. Blood urea nitrogen (BUN) test is usually done to assess the functioning of kidneys and monitor progression and treatment of kidney disease (Edgar, 2012; WebMD, 2012; Summer, 2012).

High levels of BUN in the system may be due to kidney disease, urinary tract obstruction, congestive heart failure and excessive protein levels in the gut (due to high protein diet). It may also be due to gastrointestinal bleeding, fever, metallic poisoning, pneumonia, administration of cortisol-like steroids and stressful situations (David, 2013; Summer, 2012) as well as increased protein catabolism, hypovolemia (dehydration) and antibiotics that enhance protein catabolism (e. g. tetracyclins). Whereas low levels of BUN may be

due to liver disease or injury, low protein diet, malnutrition, hypervolemia (overhydration), late pregnancy and amyloidosis (David, 2013; Edgar, 2012; Deepak *et al.*, 2007; Papadakis and Arieff, 1987).

1.3.4.2 Creatinine levels

Creatinine is formed from creatinine phosphate, a high energy molecule used by skeletal muscle (Nelson and Cox, 2005). Creatinine is a waste product of metabolism and thus, is not known to have any physiological function (Klaassen, 2008). The production of creatinine depends on the muscle mass, age, sex and race as well as amount of protein in diet (Klaassen, 2008; Nelson and Cox, 2005).

The kidneys excrete creatinine, thus, measurement of creatinine levels is used to evaluate renal function and monitor progression and treatment of kidney disease (Klaassen, 2008; Markus and Rima, 2000). Levels of creatinine in the urine and serum can be used to monitor the creatinine clearance from the body which is an indicator of glomerular filtration rate (Klaassen, 2008). High levels of creatinine are found in renal dysfunction and reduced renal blood flow (as in the case of shock, dehydration, congestive heart failure, diabetes) whiles decreases levels are found in muscular dystrophy (Klaassen, 2008; Nelson and Cox, 2005; Markus and Rima, 2000).

The measure of creatinine for renal function is superior to measurement of urea. Urea is influenced substantially by non – renal factors like high protein turn over (like diet or endogenous sources) and dehydration (Klaassen, 2008; Jacob, 2003).

1.4 THE LIVER

1.4.1 Structure of the liver

The liver is reddish brown in color (Saladin, 2003) and located in the upper right and center of the abdominal cavity, immediately beneath the diaphragm (Scanlon and Sanders, 2007; Saladin, 2003). It is the body‟s largest internal organ and weighs about 1.3 kg in an adult. It is divided into four lobes and has two ligaments (Van De Graaff, 2001). Anteriorly, the falciform ligament separates the liver into right and left lobes. Inferior view of the liver also reveals the caudate lobe located near the inferior vena cava and the quadrate lobe positioned adjacent the gall bladder. The falciform ligament holds the liver to the abdominal wall and the diaphragm. The round ligament (ligamentum teres) extends from the falciform ligament to the umbilicus and carries blood from the umbilical cord to the liver of a foetus (Saladin, 2003; Van De Graaff, 2001).

The structural and functional unit of the liver is the liver lobule (Scanlon and Sanders, 2007; Van De Graaff, 2001). The liver contains innumerable of these lobules. A lobule is made of a central vein which is surrounded by cuboidal cells (hepatocytes) and it measures about 2 mm long and 1 mm in diameter (Saladin, 2003). The central veins from several liver lobules join to form hepatic vein which transports blood from the liver to the inferior vena cava (Van De Graaff, 2001). The hepatic artery conveys oxygenated blood to the liver whereas the hepatic vein brings blood from digestive organs and spleen (Scanlon and Sanders, 2007; Saladin, 2003). The hepatocytes, which are one to two cells thick, have blood – filled channels called hepatic sinusoids. These sinusoids separate the hepatocytes from the bloodstream, and allow blood plasma into the hepatocytes and endothelium.

The sinusoids also have phagocytic cells called Kupffer cells, which remove bacteria and debris from the blood (Scanlon and Sanders, 2007). Besides, the hepatocytes produce bile which they secrete into the bile canaliculi and transported through the bile duct to the gall bladder for storage (Van De Graaff, 2001).

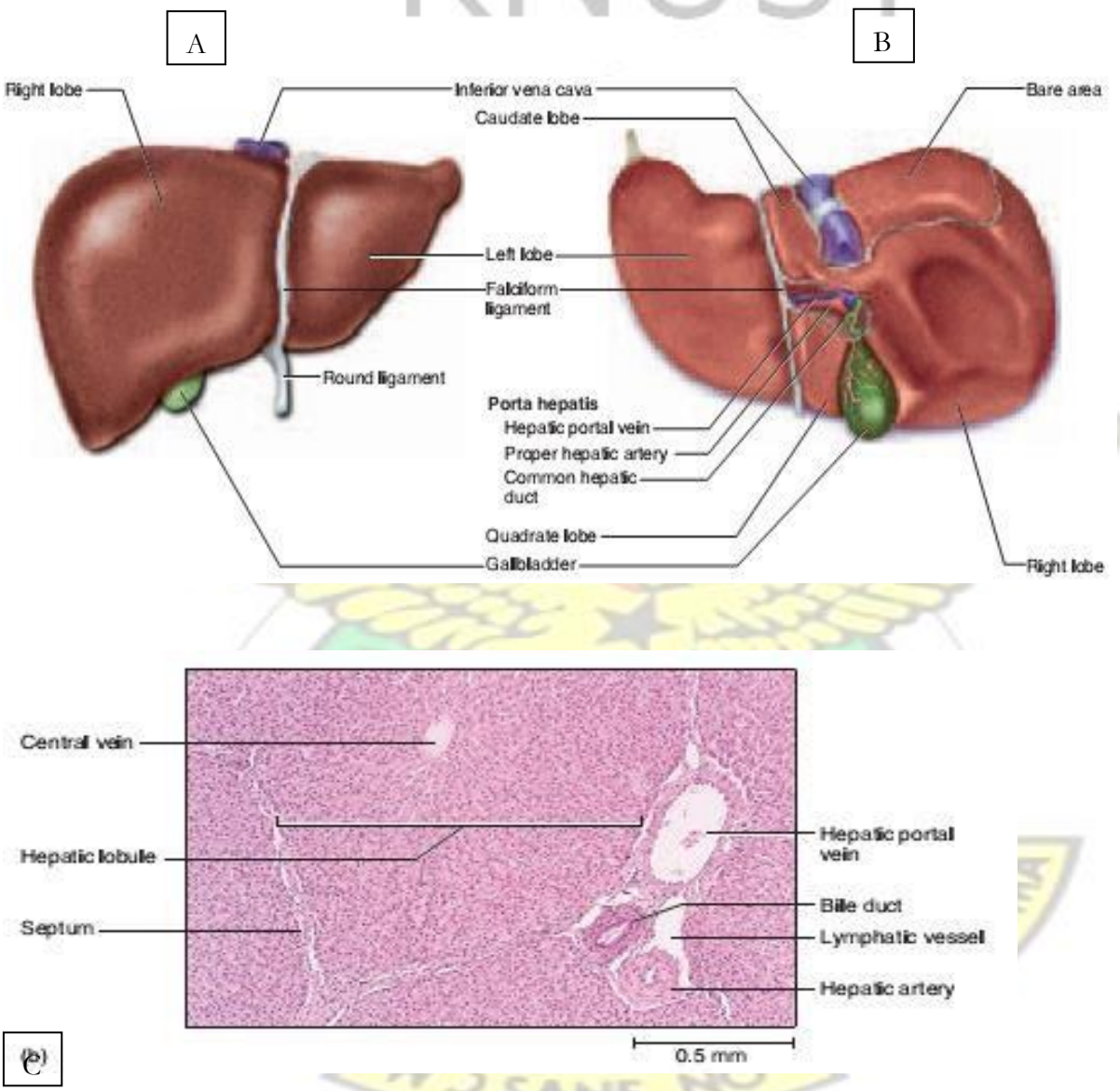


Figure 1.3: Structure of the liver (A) anterior aspect, (B) inferior aspects and (C) microscopic structure of the liver.

Source: Adapted from Saladin (2003).

1.4.2 Functions of the liver

The liver has numerous functions including synthesis, storage and release of substances as well as phagocytosis of old red blood cells and bacteria, production of bile and detoxification of xenobiotics (Klaassen, 2008; Van De Graaff, 2001; Luis *et al.*, 2003).

Liver regulates blood glucose levels by facilitating the conversion of glucose to glycogen for storage when there is excess glucose in the blood and also converts glycogen back to glucose during hypoglycemic or stressful conditions (Scanlon and Sanders, 2007). It also serves as a storage organ for iron, copper and vitamins A, B_{12}, D, E and K (Saladin, 2003).

The liver also synthesizes amino acids, plasma proteins (e.g. albumin), cholesterol and clotting factors (e.g. prothrombin) (Scanlon and Sanders, 2007; Van De Graaff, 2001). It excretes excess cholesterol into the bile for elimination in the faeces. Bile formed from the liver is used to emulsify fats and lipoproteins (also from the liver), transports the fats from the blood into other tissues (Luis *et al.*, 2003; Saladin, 2003).

Besides, the liver has Kupffer cells which phagocytize and destroy old red blood cells (RBCs) and bacteria. The RBCs are converted to bilirubin and excreted into the bile for elimination. It also synthesizes certain enzymes (e.g. cytochrome P450) which are involved in the metabolism and detoxification of xenobiotics (Klaassen, 2008; Scanlon and Sanders, 2007; Luis *et al.*, 2003).

1.4.3 Toxicity to the liver

Susceptibility of the liver to destruction by toxic substances results from the liver‟s blood supply, position in the body, its ability to metabolize toxicants and the presence of

biochemical pathways (Hartmut *et al.,* 2001; Klaassen, 2008). Its principal function is for the maintenance of homeostasis and metabolism of xenobiotics in the body, thus, metabolites produced may accumulate and cause toxic effects (Aashish *et al.,* 2013; Rolf, 2009). It is located between systemic circulation and the gastrointestinal tract to maximize absorption of nutrients and minimize the body‟s exposure to toxins, hence exposing it to high amounts of toxic substances (Jussi *et al.,* 2006). It also receives 25 % of cardiac output, exposing it to relatively high amount of toxins absorbed from digestion in the gut (Klaassen, 2008).

Poisonous effect of substances to the liver is termed as hepatotoxicity (Aashish *et al.,* 2009). Overdose of drugs or therapeutic levels of some drugs may induce liver injury (Rolf, 2009). Data on adverse drug reactions show that drug – induced liver damage accounts for 7 % of reported cases (Jussi *et al.,* 2006) with more than 900 medicines, chemicals and herbal preparations shown to induce hepatotoxicity (Aashish *et al.*, 2009).

1.4.3.1 Mechanism of hepatotoxicity

Systemic circulation transports ingested substances directly into the liver, a process termed “first pass effect” (Aashish *et al.,* 2009; Klaassen, 2008). Liver metabolizing enzymes through a series of phases convert these substances into their inactive or biologically active forms for excretion (Rolf, 2009; Kurtovic and Riordan, 2003; Hartmut *et al.,* 2001). The activities of these enzymes are influenced by the health and nutritional status of the individual, hormones, sex, age and presence of certain xenobiotics (Klaassen, 2008).

Hepatotoxicity may be due to direct toxicity of the chemical, its metabolite or from an immunologic response (Aashish *et al.,* 2009; Klaassen, 2008; Jussi *et al.,* 2006). In most instances, the actual mechanism of hepatotoxicity is unclear (Jussi *et al.,* 2006). Some compounds (e.g. aminoglycosides) also induce liver injury indirectly by promoting the production of reactive oxygen species which attack and deplete cellular structures, leading to their toxicity (Al – Kenannay *et al.,* 2012; Noorani *et al.,* 2010).

1.4.4 Assessment of liver function

Levels of hepatic enzymes and metabolites are usually evaluated to monitor liver function. These include: alanine aminotransferase (ALT, formerly; serum glutamate pyruvate transaminase, SGPT), aspartate aminotransferase (AST, formerly; serum glutamic oxaloacetic transaminase, SGOT), alkaline phosphatase (ALP), gamma – glutamyl transferase (GGT) and bilirubin (Klaassen, 2008; Nelson and Cox, 2005). Elevated levels of these markers indicate liver dysfunction (Al – Kenannay *et al.,* 2012; Noorani *et al.,* 2010).

High levels of ALT is a more specific indicator of hepatic injury than that of AST, because high levels of AST may also be symbolic of muscle, heart or kidney injury (Jussi *et al.,* 2006; Dufour *et al.,* 2000). Elevated levels of ALP, GGT and bilirubin are indicative of compromised liver function and cholestasis (Larrey, 2002; Williams and Iatropoulos, 2002).

1.5 BIOMARKERS FOR ASSESSING CELLULAR INJURY

Aerobic cells depend on oxygen as the final acceptor of electrons in respiration. This allows them to extract more energy from food than would be possible without oxygen and consequently generating reactive oxygen species (ROS) or free oxygen radicals (Goodsell, 2007). Free radicals and related species are produced from oxygen (ROS) and nitrogen (reactive nitrogen species/ RNS). These are usually produced in the body from normal metabolism or from effect of external sources like pollution, cigarette smoke, stress, radiation and medication, on the body (Sivanandham, 2011). Examples of reactive species include: singlet oxygen, hydrogen peroxide, superoxide anion, nitroxides and hydroxyl radicals (Sarma *et al.*, 2010).

Though ROS are produced by normal aerobic cells, their physiologic role depends on their levels in the cell (Weydert and Cullen, 2009). The accumulation of these reactive species leads to oxidative stress (Sivanandham, 2011). They are shown to have detrimental impact on cell constituents and hence result in cellular destruction and dysfunction (Gholamreza *et al*., 2005; Ezzat, 1996). Several evidences also show that free radicals and decrease in antioxidant activity are implicated in xenobiotics – induced nephrotoxicity, rheumatoid arthritis, cataract, cancer, artherosclerosis, aging, cadiovascular and neurodegenerative disorders (Aslam *et al.*, 2013; Kumar *et al.*, 2013; Pedraza-Chaverri *et al.*, 2008; Niki *et al.*, 2005).

The human body utilizes several means (e.g. reduced glutathione, superoxide dismutase, catalase and others) for counteracting oxidative stress (Aslam *et al.*, 2013; Sivanandham, 2011).

1.5.1 Reduced glutathione

Glutathione is a water soluble cellular compound with cysteine, glutamine and glycine as its components (Townsend *et al.*, 2003). It is a vital component of cells throughout the body for cellular maintenance and survival (Main *et al.*, 2012). It also functions as a co – factor for signal transduction, a redox buffer, antioxidant and electrophile defence (Johnson *et al*., 2012; Townsend *et al*., 2003). The relevance of reduced glutathione (GSH) is manifested by the extensive usage in some prokaryotes, animals, plants and fungi (Townsend *et al*., 2003). GSH has a role in detoxification of various compounds in reactions involving glutathione peroxidases (GPx) and glutathione-S-transferases (GST) (Johnson *et al*., 2012; Townsend *et al*., 2003).

The thiol group on GSH is a potent reducing agent in some tissues (Townsend *et al*., 2003). When glutathione is oxidized, glutathione reductase uses NADPH as a co-factor to convert the oxidized glutathione (glutathione disulphide, GSSG) to its reduced form (GSH). The ratio of GSH to GSSG in a cell or tissue is used to assess toxicity within the cell or tissue (Johnson *et al*., 2012; Townsend *et al*., 2003). Insufficient amounts of GSH within a cell predispose the cell to damage by oxidative stress (Aslam *et al*., 2013). An inadequate amount of GSH in tissues has been implicated in various disease conditions including: renal injury, carcinogenesis, liver damage, pancreatitis and neurodegenerative diseases (Johnson *et al*., 2012; Townsend *et al*., 2003).

1.5.2 Superoxide dismutase (SOD)

It has been shown that superoxide radicals are implicated in various disease conditions (Christos *et al.*, 2008). Superoxide ion is produced from different sources like normal

cellular respiration, activated polymorphonuclear cells, mitochondria electron flux and endothelial cells (Lenaz, 2001; McCord and Omar, 1993).

SOD also known as superoxide oxidoreductase (EC 1.15.1.1) is an enzyme involved in the catalysis of superoxide radicals to oxygen and hydrogen peroxide (Abreu and Cabelli, 2009). It constitutes the first line of defence against reactive oxygen species (Abreu and Cabelli, 2009; Larry and Garry, 1979). Superoxide dismutase (SOD) is believed to be absent in most obligate anaerobes but existent in all aerobic cells. This may be due to its physiological role in providing defence against potential damage by superoxide radical produced from aerobic metabolic reaction (Larry and Garry, 1979).

Research has established that low levels of superoxide dismutase have been found in tissues prone to oxidative stress (Stevens *et al.*, 2000; Larry and Garry, 1979). Several studies have also shown that low levels of SOD are observed in pulmonary disease (Khan, 2012), nephrotoxicity (Aslam *et al*., 2013), hepato-renal injury (Adejuwon *et al*., 2014), hyperglycemia (Johnny *et al.*, 2014) and inflammation (Larry and Garry, 1979).

1.5.3 Catalase

Hydrogen peroxide is produced from a number of oxidative reactions in the cell. It however has a detrimental effect on cellular structures like proteins, lipids and DNA and therefore should be quickly removed from the body (Eboh *et al.*, 2013; Sivanandham, 2011; Weydert and Cullen, 2009).

To this effect, catalase (EC 1.11.1.6) is frequently used by cells to convert hydrogen peroxide to less reactive products – oxygen and water (Sivanandham, 2011; Weydert and Cullen, 2009). It is found in most living cells but abundant in liver, kidney and blood (Klaassen, 2008). Catalase also utilizes peroxide to detoxify various metabolites and toxins like alcohol, formaldehyde, phenols, acetaldehyde and formic acid (Sivanandham, 2011; Klaassen, 2008).

Several studies have shown that catalase levels are low during nephrotoxicity (Aslam *et al*., 2013), hepato-renal injury (Adejuwon *et al*., 2014), inflammation (Larry and Garry, 1979), hyperglycemia (Johnny *et al.*, 2014) and pulmonary disease (Khan, 2012).

1.5.4 Lipid peroxidation

Lipid peroxidation is a natural metabolic process that takes place in both plants and animals (Repetto *et al.*, 2012; Gago – Dominguez *et al.*, 2007; Janero, 1990). It is defined as an oxidative degradation of lipids and other lipid – containing structures (Girotti, 1998). In the process of lipid peroxidation, reactive species attract electrons from lipids of the cell membranes, leading to cell damage and eventually cell death (Gago – Dominguez *et al*., 2007; Girotti, 1998). It mostly affects unsaturated lipids especially polyunsaturated fatty acids (PUFA) (Repetto *et al.*, 2012). Products of lipid peroxidation (e.g. alcohols, ketones, aldehydes, alkane and esters) are known to have various roles in the signal transduction cascade, the control of cell proliferation, and the induction of differentiation, maturation and apoptosis (Barrera *et al.*, 2004; Cejas *et al.*, 2004).

However in pathological situations, the reactive oxygen and nitrogen species are generated at higher than normal rates, and as a consequent, there is increased destruction of cellular structures and death of cells (Repetto *et al.*, 2012; Barrera *et al.*, 2004). The quantification of lipid peroxidation is therefore necessary to assess the role of oxidative injury in pathophysiological conditions (Repetto *et al.*, 2012). Thus, lipid peroxidation is assessed in relation to the amount of malondialdehyde (MDA) produced. Malondialdehyde is a byproduct from the peroxidation of lipids, it combines with several functional groups on molecules including lipoproteins, DNA and proteins and it helps to visualize the MDA – adduct formed (Janero, 1990).

1.5.5 Myeloperoxidase (MPO)

Myeloperoxidase (EC 1.11.1.7) is a heme-linked peroxidase present in macrophages, monocytes and neutrophils (most abundant in neutrophils) and plays a vital role in immune surveillance and defence (Galijasevic, 2013). Upon leukocytes activation, MPO is released to use NADPH in converting hydrogen peroxide and chloride ions to hypochlorous acid (HOCl) and other highly reactive products (Amy *et al.*, 2014; Metzler *et al.*, 2011). These reactive products are microbicidal in nature (Galijasevic, 2013; Metzler *et al.*, 2011). The products also react with lipids, proteins, lipoproteins and DNA to influence signalling pathways and induce protein adducts and genetic mutations (Amy *et al.*, 2014; Galijasevic, 2013).

Myeloperoxidase is involved in various processes of tissue damage and elevated levels of free MPO are observed in tissues sites with inflammation (Arnhold, 2004). Thus, high MPO

activity has been linked to a number of pathologies including rheumatoid arthritis, kidney damage, lung disease, cancer, atherosclerosis and neurogenerative diseases (Galijasevic, 2013; Arnhold, 2004; Anitra *et al.*, 2000).

1.6 JUSTIFICATION FOR THE PRESENT STUDY

Nephrotoxicity is a common and important side effect of various drugs and this limits their clinical relevance and is a critical complication of therapeutics (Rajitha *et al.*, 2013). Several medications have been used for the treatment of kidney diseases, of which some are; Allopurinol (for treatment certain types of kidney stones), dopamine IV and dopamine in D5W IV (for acute kidney diseases) (WebMD, 2013); and several antihypertensive drugs such as furosemide, chlorothiazide, propranolol, Losartan, Valsartan and Amiloride (William *et al.*, 2013; Zillich *et al*., 2006).

Liver diseases on the other hand, account for 5 % of all hospitalized cases and 75 % of adverse drug reactions resulting in death in the United States (Sivakrishnan and Kottaimuthu, 2013). It is a common cause of drug withdrawal from the market (Hartmut *et al.*, 2001; Rolf, 2009). Various medicines are used for the management of liver conditions.

Examples include: prednisolone for alcoholic hepatitis, silymarin for cirrhosis (Luis *et al.*, 2003), gamma globulin for the prevention of hepatitis A (Scanlon and Sanders, 2007) as well as propranolol and nadolol for the management of variceal hemorrhage (Guadalupe and Joseph, 2009).

However, these medications used for the treatment of kidney and liver diseases are usually associated with side effects such as increased risk of new-onset diabetes (Zillich *et al*., 2006), headache, nausea, hyponatremia, low blood pressure, chest pain and emotional / behavioral changes (WebMD, 2013). There is therefore the need to search for alternative ways of preventing and/ or treating kidney and liver diseases with fewer side effects.

Plant extracts and phytochemicals are very beneficial in the treatment of various diseases and conditions (Varalakshmi *et al*., 2011). Example of such plant is *Terminalia ivorensis. Terminalia ivorensis* is a deciduous plant used medicinally for ulcers, cuts, sores, wounds, general body pains, hemorrhoids, diuretics, malaria and yellow fever (Ouattara *et al.*, 2013; Akinyemi *et al.*, 2006; Etukudo, 2003; Burkill, 1985). Due to its diuretic (Akinyemi *et al.*, 2006), anti-hyperglycemic (Johnny *et al.,* 2014) and anti – inflammatory properties (Iwu and Anyanwu, 1982), the present study was aimed at exploring the nephroprotective and hepatoprotective effects of *Terminalia ivorensis* extract (TIE) in Sprague – Dawley rats.

This study specifically investigated the possible kidney and liver protective effects of TIE against oxidative stress, liver dysfunction and renal impairment induced by gentamycin and potassium dichromate. The findings from this work are expected to add value to the overall usage of medicinal plants especially *Terminalia ivorensis.*

1.7 AIM AND SPECIFIC OBJECTIVES

1.7.1 Aim:

To assess the nephroprotective and hepatoprotective effects of *Terminalia ivorensis* against potassium dichromate and gentamycin induced toxicities in SD rats and the possible mechanism of protection.

1.7.2 Specific objectives of the study;

- To assess the effect of extract on renal parameters due to nephrotoxicity induced by potassium dichromate and gentamycin in Sprague Dawley rats by measuring:
  - Urine output
  - Organ and body weights
  - Urea
  - Creatinine
  - Serum electrolytes (sodium, potassium and chloride)
- To assess the effect of extract on markers of tissue injury due to nephrotoxicity induced by potassium dichromate and gentamycin in Sprague Dawley rats by estimating the levels of:
  - Reduced glutathione
  - Superoxide dismutase
  - Catalase
  - Lipid peroxidation
  - Myeloperoxidase
  - Kidney histomorphology
- To assess the effect of extract on biomarkers of liver injury due to hepatotoxicity induced by gentamycin in Sprague-Dawley rats by the determination of:
  - Organ and body weights
  - AST, ALT, GGT, ALP and total bilirubin
  - Total protein, globulin and albumin

- Liver histology

CHAPTER TWO

MATERIALS AND METHODS

2.1 PREPARATION OF PLANT MATERIAL

Stem bark of *Terminalia ivorensis* was collected from Asakraka Kwahu in the Eastern region of

Ghana in September 2014. The sample was verified at the Department of Pharmacognosy, KNUST, Ghana, and given a voucher herbarium number FP/095/10 as well as preserved in the same department.

The stem bark was washed with water, air dried, made into powder with a laboratory blender and weighed. Ethanol (70 %) was used for the extraction of the sample by adding 5 litres to 1 kg of the powdered sample and leaving it for 72 hours. The supernatant was filtered and concentrated using a rotary evaporator. The sample was then dried at 55 ^{O}C in an oven. The yield obtained was 24.3 % w/w. The extract was then stored in a refrigerator.

The extract was prepared for dosing by dissolving it in distilled water which was also used as vehicle for the control groups throughout the studies. The concentrations of the extract were prepared such that no animal received more than 1.0 mL/kg body weight. The samples were administered to the animals orally by gavage.

2.2 ANIMALS

Sprague Dawley rats (160 – 240 g) of both sexes were used for the experiments. They were acquired from the Animal house of the Department of Pharmacology, KNUST. The animals were allowed to acclimatize for a week before an experiment and were given free access to feed and water. All experimental animals were housed in aluminium laboratory cages (34 x 47 x 18 cm) with wood shavings as bedding.

2.3 CHEMICALS AND DRUGS

Ethanol, glacial acetic acid, n-butanol, hydrogen peroxide and Tris – HCl, were obtained from BDH chemicals Ltd Poole, England. Phenazine methosulphate 5,5‟ – dithiobis-(2-nitrobenzoic acid), o-dianisidine, nitroblue tetrazolium (NBT) reduced nicotinamide adenine dinucleotide (NADH), thiobarbituric acid (TBA), trichloroacetic acid (TCA) and potassium dichromate were acquired from Sigma chemicals Co., St Louis, Missouri, USA and gentamycin (Rooche Pharmaceutical Ltd, China).

2.4 PHYTOCHEMICAL ANALYSES

Phytochemicals are bioactive components of plants which are not nutrients but very beneficial to the plants. Some bioactive components analyzed from the crude stem bark extract qualitatively included flavonoids, alkaloids, steroids, condensed tannins, saponins, glycosides, terpenoids and anthraquinones. The methods used were as described by Sofowora, (1982).

2.4.1 Test for flavonoids

A volume of dilute ammonia (2 mL) was added to the extract (0.5 g) and concentrated H_2SO_4 was also added.

2.4.2 Test for alkaloids

A mass of extract (0.5 g) was added to 1 % w/v dilute sulphuric acid (5 mL) on a steam bath. The solution was filtered and the filtrate treated with a few drops of Dragendorff‟s reagent.

2.4.3 Test for steroids

Extract (0.5 g) was dissolved in 2 mL of chloroform and filtered. Acetic acid anhydride (2 mL) was added to 2 mL of the filtrate and 2 mL concentrated H_2SO_4.

2.4.4 Test for tannins

Extract (0.5 g) was dissolved in 10 mL distilled water. This was filtered and the filtrate treated with 1 % ferric chloride.

2.4.5 Test for saponins

A volume of 5 mL distilled water was added to the extract (0.5 g) and shaken vigorously. The content was then warmed over water bath.

2.4.6 Test for terpenoids

A mass of extract (0.5 g) was mixed with chloroform (2 mL) and filtered. Three drops of concentrated H_2SO_4 was then carefully added to the filtrate to form a thin layer.

2.4.7 Test for cardiac glycosides

A volume of dilute H_2SO_4 (2 mL) was added to the extract (0.5 g) and filtered. Potassium hydroxide (20 %) was added to the filtrate until it became alkaline. The alkalinity of the filtrate was confirmed with a red litmus paper which turned blue. Fehling"s solutions A and B were also added and heated over a warmed water bath.

2.4.8 Test for anthraquinones

The extract (0.5 g) was added to 5 mL dilute H_2SO_4 and heated for 5 minutes over a water bath. This mixture was filtered and chloroform (2 mL) was added to the filtrate and 2 mL of dilute ammonia was also added.

2.5 LD_{50} AND ACUTE TOXICITY IN RATS

The method used for the acute toxicity study was as described by Amna *et al.* (2013). Thirty Sprague – Dawley rats (160 – 200 g) of both sexes were put into six groups (n =5) and were allowed to acclimatize with their environment for a week with free access to water and feed. Group I (control) was given distilled water as vehicle and groups II – VI received 100, 500, 1000, 3000 and 5000 mg/kg of extract orally by gavage respectively. After treatment, the rats were observed for 24 hours at 30 minutes intervals for general behavioral, pharmacological changes and death. The changes assessed were hypoactivity, aggressiveness, drinking, vomiting, diarrhea, excitement, eating, sedation and death. The animals were observed for 14 days for possible toxicity of the extract.

2.6 EFFECT OF EXTRACT ON POTASSIUM DICHROMATE – INDUCED NEPHROTOXICITY

The method used was as described by Aslam *et al.* (2013).

Thirty (30) Sprague – Dawley rats (180 – 240 g) of both sexes were weighed, put into five groups (n =6) and left to acclimatize for a week with adequate fresh water and feed.

SD rats were grouped and drugs administered as shown below:

- Group I: control (distilled water 1 mL/kg/day orally) for 5 days.
- Group II: distilled water (1 mL/kg/day orally) for 5 days.
- Group III: extract (100 mg/kg/day orally) for 5 days.
- Group IV: extract (300 mg/kg/day orally) for 5 days.
- Group V: extract (1000 mg/kg/day orally) for 5 days.

On the fourth day, $K_2Cr_2O_7$ (20 mg/kg) was injected subcutaneously to all animals except group I and all animals were put in metabolic cages for collection of 24 – hour urine.

On day 6, all animals were weighed and blood samples collected for estimation of serum electrolytes, blood urea nitrogen and creatinine levels. All animals were then sacrificed and their kidneys removed, washed with normal saline and weighed. The right kidneys were preserved for histopathological studies while left kidneys were used for post – mitochondrial supernatant. The change in body weight was expressed in terms of percentage.

2.7 EFFECT OF EXTRACT ON GENTAMYCIN – INDUCED NEPHROTOXICITY

The method used was as described by Qadir *et al.* (2011).

Thirty (30) male SD rats (180 – 240 g) were weighed, put into five groups (n =6) and left to acclimatize for a week with adequate supply of fresh water and feed.

Animals were put into groups and drugs administered as described below:

- Group I: control (distilled water 1 mL/kg/day orally) for 14 days.
- Group II: gentamycin (80 mg/kg/day i.p.) for 14 days.
- Group III: extract (100 mg/kg/day orally) + gentamycin (80mg/kg/day i.p.) for 14 days.
- Group IV: extract (300 mg/kg/day orally) + gentamycin (80mg/kg/day i.p.) for 14 days.
- Group V: receive extract (1000 mg/kg/day orally) + gentamycin (80mg/kg/day i.p.) for 14 days.

Treatment of animals with the extract for groups III, IV and V started 3 days prior to the commencement of the study. Injection of gentamycin was done at 10:00 hours to minimize the circadian variation in nephrotoxicity.

On day 14, all animals were put in metabolic cages for collection of 24 – hour urine. On the 15th day, all animals were weighed and blood samples collected for estimation of serum electrolytes, blood urea nitrogen and creatinine levels. All animals were then sacrificed and their kidneys removed, washed with normal saline and weighed. The right kidneys were preserved for

histopathological studies while left kidneys were used for post – mitochondrial supernatant. The change in body weight was expressed in terms of percentage.

2.8 GENTAMYCIN – INDUCED HEPATOTOXICITY

The method used was as described by Noorani *et al.* (2010).

Thirty (30) SD rats (180 – 240 g) were weighed, put into five groups (n =6) and left to acclimatize for a week with adequate supply of fresh water and feed.

Animals were grouped and drugs administered as follows:

- Group I: control (distilled water 1 mL/kg/day orally) for 7 days.
- Group II: gentamycin (80 mg/kg/day i.p.) for 7 days.
- Group III: extract (100 mg/kg/day orally) + gentamycin (80mg/kg/day i.p.) for 7 days.
- Group IV: extract (300 mg/kg/day orally) + gentamycin (80mg/kg/day i.p.) for 7 days.
- Group V: extract (1000 mg/kg/day orally) + gentamycin (80mg/kg/day i.p.) for 7 days.

On the 8th day, all animals were weighed and blood samples collected for estimation of liver markers. All animals were then sacrificed and their livers removed, washed with normal saline, weighed and preserved for histological studies.

EFFECT OF EXTRACT ON

2.9 SERUM BIOCHEMICAL PARAMETERS

Blood samples were centrifuged at 5000 r.p.m for 10 minutes to obtain sera which were used for the estimation of the various kidney and liver biochemical markers. Estimation of urea and creatinine were done with Biotecnica BT 3000 Targa chemistry analyzer. Whereas measurement of sodium, potassium and chloride levels were done with Flexor junior auto analyzer. Liver biomarkers were also measured with Biotecnica BT 3000 Targa chemistry analyzer.

2.10 EFFECT OF EXTRACT ON BIOMARKERS OF OXIDATIVE STRESS

For the preparation of mitochondrial – supernatant (PMS), left kidneys was quickly cleaned and put in ice – cold saline (0.85 % sodium chloride). Potter - Elvehjem homogenizer (Ultra-Turrax T25, Staufen, Germany) was used to homogenize the kidneys. The homogenization was done at 4 ^{O}C in phosphate buffer (0.1M, pH 7.4) with KCl (1.17 %).

The tissue homogenate was filtered through a clean handkerchief and centrifuged with Hettichzentrifugen (Micro 220R, Germany) at 800 X g for 5 minutes at 4 ^{O}C. The supernatant was again centrifuged at 5000 rpm for 30 minutes at 4 ^{O}C to obtain the PMS which served as the enzyme source for the assessment of markers of oxidative stress. All the assays were done within 24 hours after animal sacrifice (Aslam *et al.,* 2013).

EFFECT OF EXTRACT ON

2.10.1 Estimation of reduced glutathione (GSH) levels

The estimation of GSH levels was done as described by Ellman (1959). In this method, thiol groups react with 5,5‟ – dithiobis-(2-nitrobenzoic acid) to form a colored anion which peaks at 412 nm.

Aliquots 0.l mL of post – mitochondrial supernatant (PMS) were added to 2.4 mL EDTA (0.02 M) solution and kept on ice bath for 10 minutes. Volumes of 2 mL distilled water and 0.5 mL trichloroacetic acid (TCA, 50 %) were then added and the mixture centrifuged at 3000 X g for 20 minutes at 4 ^{O}C to take out precipitate. Aliquots of supernatant (1 mL) were then mixed with 0.05 mL of 5,5‟ – dithiobis-(2-nitrobenzoic acid (10 mM, DTNB) and 2.0 mL of Tris – HCl buffer (0.4 M, pH 8.9), which was vortexed thoroughly and incubated for 5 minutes at room temperature. Absorbance was read at 412 nm against a reagent blank without homogenate after the addition of DTNB. Absorbance was measured in the visible range using Synergy H1 Hybrid plate reader (Biotek, JosHansen). The glutathione values were extrapolated from a standard glutathione graph and expressed as µM/mg of protein.

2.10.2 Assessment of superoxide dismutase activity

Superoxide dismutase activity was estimated as described by Sun *et al.* (1988). This method is based on inhibiting the reduction of nitroblue tetrazolium (NBT) by superoxide radical. One unit of the enzyme was termed as the quantity of enzyme causing 50 % inhibition in the reduction rate of NBT. SOD activity was stated as U/mg of protein.

A volume of 0.5 mL PMS was added to 0.4 mL mixture of ethanol and chloroform, and the reaction mixture centrifuged at 5000 r.p.m. for 5 minutes at 4 ^{O}C. Phenazine methosulphate (0.1 mL), 1.0 mL sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 mL nitroblue tetrazolium and

0.1 mL reduced nicotinamide adenine dinucleotide (NADH) were added to the supernatant and incubated for 3 minutes at 30 ^{O}C. About 2.0 mL mixture of glacial acetic acid and n-butanol was used to stop the reaction. The absorbance was measured at 560 nm with Synergy H1 Hybrid plate reader (Biotek, JosHansen).

The enzyme activity was calculated with the following equations:

$$\text{Rate (R)} = \frac{\text{final absorbance - initial absorbance}}{\text{3 minutes}}$$

$$\% \text{ Inhibition} = \frac{\text{Absorbance of blank} - R}{\text{Absorbance of blank}} \times 100$$

$$\text{1 Enzyme unit (U)} = \frac{\% \text{ Inhibition}}{50} \times \text{common dilution factor}$$

50% inhibition =1 enzyme unit

$$\text{Specific activity (units/mg protein or tissue)} = \frac{\text{activity in units of enzyme}}{\text{mg protein}}$$

2.10.3 Assessment of catalase levels

Catalase activity was estimated as described by Sinha (1972). The degradation of hydrogen peroxide was measured at 620 nm.

The reaction mixture was made of 0.4 mL of 2.0 M hydrogen peroxide, 1.0 mL phosphate buffer (0.01 M, pH 7.0) and 0.1 mL of PMS. After incubating the mixture at room temperature for 5 minutes, 1.5 mL of 5 % $K_2Cr_2O_7$ – glacial acetic acid mixture (1:3) was added to terminate the reaction. The absorbance was measured at 620 nm. One unit of enzyme was defined as the quantity of enzyme required to decompose 1 mmol of H_2O_2 per minute. Results were stated as U/mg of protein.

Moles of H_2O_2 consumed/min (units/mg) = $2.3/\Delta t$ x ln (ABS initial /ABS final) x $1.63x\ 10^{-3}$

ABS = Absorbance at 620 nm, Δt = time required for a decrease in the absorbance.

2.10.4 Assessment of lipid peroxidation

Peroxidation of lipids in kidney homogenate was expressed in relation to malondialdehyde formed. Amount of MDA produced was estimated as described by Ohkawa *et al.,* (1979). A volume of 0.2 mL of tissue homogenate was added to 1.5 mL of acetic acid (20 %), 0.2 mL of SDS (8.1 %) and 0.8 mL of TBA (0.8 %). distilled water was used to make the total volume to 4.0 mL and the mixture heated at 95 ^{O}C for 60 minutes in a water bath. This was left to cool to room temperature, and 5.0 mL n-butanol and 1.0 mL distilled water were added. The mixture was vortexed vigorously, and then centrifuged for 10 minutes at 4000 r.p.m. The absorbance of the organic layer was read at 532 nm. The procedure was repeated for 1,1,3,3tetramethoxypropane as standard. The level of lipid peroxidation measured was stated as µM MDA/g of tissue.

2.10.5 Estimation of myeloperoxidase (MPO) levels

The method used for the estimation of myeloperoxidase activity was as described by Senoglu *et al.,* (2009).

Myeloperoxidase level was estimated with O – dianisidine method. The reaction mixture contained 0.3 mL of 0.01 M H_2O_2, 0.3 mL of 0.1 M phosphate buffer (pH 6.0), 0.5 mL 0.02 M o-dianisidine in deionized water and 10 µL tissue homogenate making a volume of 3.0 mL with deionized water. The tissue homogenate was added last. The change in absorbance at 460 nm was followed every minute for 10 minutes. All assays were carried out in duplicates. One unit

of enzyme was defined as the amount of enzyme producing an increase in absorbance of 0.001 min^{-1} and specific activity was stated as units/mg protein.

The enzyme activity was calculated the equations below:

Rate (R) = final absorbance - initial absorbance

10 minutes

1 Enzyme unit (U) = R/ 0.001

Specific activity (units/mg protein or tissue) = activity in units of enzyme

mg

protein

2.10.6 Histopathological examination of kidney and liver

Immediately after sacrifice, right kidneys of rats were stored in neutral buffered formalin (10 %) for histological processing. Sections of the kidneys and livers were stained with hemotoxylin and eosin, and observed under a Leica DM 750 microscope (ICC50 HD, JosHansen, Germany) at magnifications of X 400 for kidneys and X100 for livers.

2.11 STATISTICAL ANALYSIS

GraphPad Prism 6.0 was used for the analyses and differences in comparing groups were done with One – Way Analysis of Variance (ANOVA) followed by Sidak‟s multiple comparison test. All results were expressed as mean ± standard error of mean (SEM) and ($p < 0.05$) was considered significant.

CHAPTER THREE

RESULTS

3.1 PHYTOCHEMICAL ASSESSMENT

In this study, the extract was shown to possess flavonoids, steroids, alkaloids, saponins, condensed tannins, cardiac glycosides, terpenoids and anthraquinones (Table 3.1). This was in agreement with earlier findings by Johnny *et al.,* (2014) and Coulibaly *et al.*, (2014). In contrast to these findings, Annan *et al.*, (2012) also reported that, the plant possesses alkaloids, saponins, tannins and flavonoids but however lacked terpenoids, steroids and glycosides.

Table 3.1: Phytochemical components of the extract

Phytochemicals	Results
Flavonoids	+
Alkaloids	+
Condensed tannins	+
Steroids	+
Saponins	+
Cardiac glycosides	+
Terpenoids	+
Anthraquinones	+

+ : means present.

3.2 LD_{50} AND ACUTE TOXICITY STUDY

The extract treated groups exhibited signs of sedation. Treatment with extract (100 - 5000 mg/kg) did not cause death in a 24 – hour period. After further observation for 14 days, treatment did not result in latent toxicity and the LD_{50} was estimated to be above 5000 mg/kg (Table 3.2).

Table 3.2: Effect of extract (TIE) on general behavior/ cage side observations

TREATMENT	OBSERVATIONS MADE IN RATS IN ACUTE TOXICITY								
	aggression	eating	excitement	salivation	diarrhea	drinking	vomiting	sedation	death
Control	-	+	-	-	-	+	-	-	-
100 mg/kg	-	+	-	-	-	+	-	+	-
500 mg/kg	-	+	-	-	-	+	-	+	-
1000 mg/kg	-	+	-	-	-	+	-	+	-
3000 mg/kg	-	+	-	-	-	+	-	+	-
5000 mg/kg	-	+	-	-	-	+	-	+	-

(-) Implies not observed, (+) implies observed

3.3 EFFECT OF *TERMINALIA IVORENSIS EXTRACT* (TIE) ON POTASSIUM DICHROMATE ($K_2CR_2O_7$) – INDUCED NEPHROTOXICITY

3.3.1 Effect of $K_2Cr_2O_7$ and TIE on urine volume

Analyses of 24 – hour urine output on the fifth day of the experiment showed that, the extract (TIE) at 300 ($p< 0.01$) and 1000 mg/kg ($p< 0.001$) significantly increased the urine volume of rats when compared to the control group (figure 3.1).

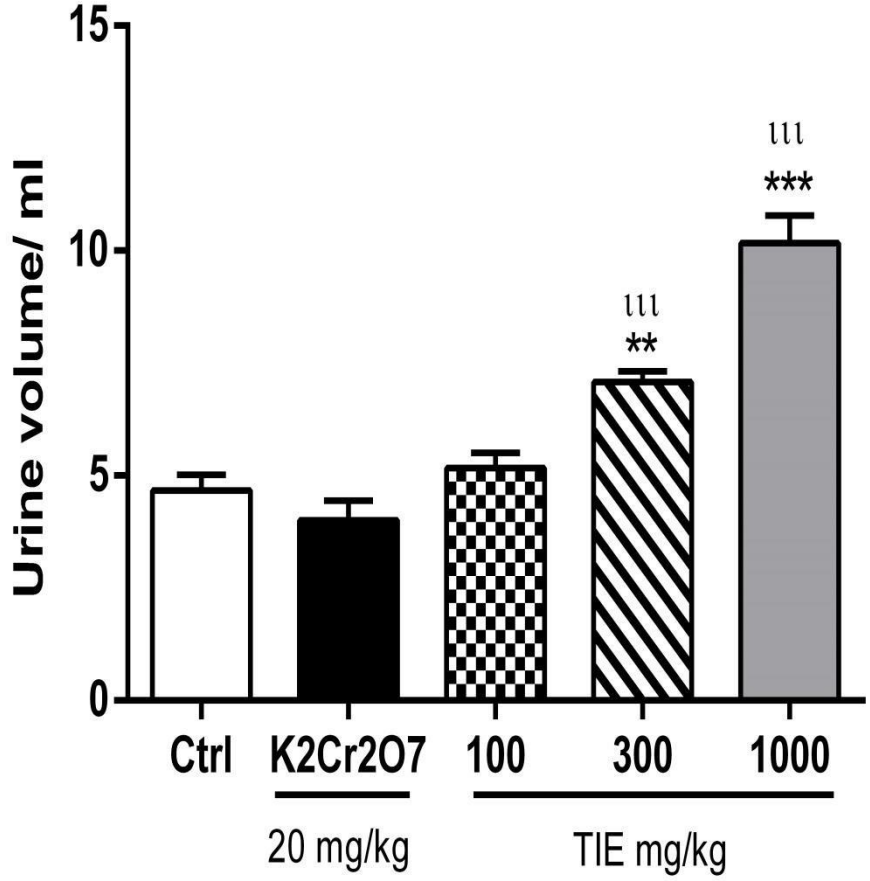


Figure 3.1: Effect of $K_2Cr_2O_7$ (20 mg/kg) and TIE (100, 300, 1000 mg/kg) on 24 – hour urine output. **p< 0.01, ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak"s post hoc test) and ^{ttt}p< 0.001 as compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak"s post hoc test).

3.3.2 Effect of $K_2Cr_2O_7$ and TIE on body and kidney weights

No significant difference was observed in the kidney weights and percent change in body weights in all groups of rats as compared to the control. The extract at 1000 mg/kg however, exhibited a significant (p< 0.05) increase in percentage change in body weight when compared to the $K_2Cr_2O_7$ group (table 3.3).

Table 3.3: Effect of TIE and $K_2Cr_2O_7$ on kidney weight and % change in body weight

Treatment group	Kidney weight /g	% Change in body weight
Control: distilled 10ml/kg	1.18 ± 0.01	0.49 ± 1.04
$K_2Cr_2O_7$ 20 mg/kg	1.33 ± 0.06	-7.13 ± 3.72
TIE 100 mg/kg + $K_2Cr_2O_7$	1.41 ± 0.10	-3.17 ± 2.07
TIE 300 mg/kg + $K_2Cr_2O_7$	1.35 ± 0.06	-1.47 ± 1.64
TIE 1000 mg/kg + $K_2Cr_2O_7$	1.35 ± 0.04	4.05 ± 2.83[t]

All data are stated as mean ± S.E.M. (n=6), [t]p< 0.05 when compared to the $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak‟s post hoc test).

3.3.3 Effect of TIE and $K_2Cr_2O_7$ on serum creatinine and urea

Significant elevation in serum creatinine levels were observed in potassium dichromate ($K_2Cr_2O_7$) and 100 mg/kg (p< 0.001) groups when compared to the control. There was again significant increase in creatinine levels in 300 (p< 0.01) and 1000 (p< 0.05) mg/kg of extract when compared to the control group. Whereas levels of creatinine were significantly lowered at 300 and 1000 mg/kg (p< 0.001) and 100 mg/kg (p< 0.05) when compared to $K_2Cr_2O_7$ group.

Significantly (p< 0.001) increased serum urea levels were observed in $K_2Cr_2O_7$ (20 mg/kg), 100 and 300 mg/kg of extract as well as in 1000 mg/kg (p< 0.01) when compared to the control group. Whereas extract (TIE), at 300 (p< 0.05) and 1000 (p< 0.001) mg/kg significantly lowered the levels of serum urea as compared to the $K_2Cr_2O_7$ group (Table 3.4).

Table 3.4: Effect of TIE and $K_2Cr_2O_7$ on serum Creatinine and urea

Treatment group	Creatinine/ µmol/L	Urea/ mmol/L
Control: distilled 10ml/kg	27.83 ± 1.85	5.09 ± 0.11
$K_2Cr_2O_7$ 20 mg/kg	358.70 ± 34.56***	56.55 ± 3.63***

TIE 100 mg/kg + $K_2Cr_2O_7$	255.50± 28.47***^{t}	53.33 ± 7.02***
TIE 300 mg/kg + $K_2Cr_2O_7$	160.50± 6.84**^{ttt}	37.81 ± 2.57***^{t}
TIE 1000 mg/kg + $K_2Cr_2O_7$	123.70 ± 4.79*^{ttt}	27.81 ± 2.24**^{ttt}

All data are stated as mean ± S.E.M. (n=6), *p< 0.05, **p< 0.01 and ***p< 0.001 when compared to control group (one way ANOVA followed by Sidak"s post hoc test). ^{t}p< 0.05 and ^{ttt}p< 0.001 when compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak"s post hoc test).

3.3.4 on serum sodium, potassium and chloride levels

$K_2Cr_2O_7$ significantly ($p< 0.01$) increased sodium and potassium levels as well as chloride levels ($p< 0.05$) when compared to the control group.

However, extract pre – treated rats at 1000mg/kg significantly ($p< 0.05$) diminished sodium levels, whereas extract at 300 and 1000mg/kg significantly reduced potassium as compared to the $K_2Cr_2O_7$ group. No significant difference was detected in the chloride levels when the extract treated groups were compared to the control and $K_2Cr_2O_7$ groups (figure 3.5).

Table 3.5: Effect of TIE and $K_2Cr_2O_7$ on serum sodium, potassium and chloride levels

Treatment group	Sodium/ m/L	Potassium/ m/L	Chloride/ m/L
Control: distilled 10ml/kg	121.70 ± 3.25	12.85 ± 0.48	84.50 ± 2.35
$K_2Cr_2O_7$ 20 mg/kg	136.30 ± 2.81**	15.70 ± 0.55**	95.67 ± 3.80*
TIE 100 mg/kg + $K_2Cr_2O_7$	129.70 ± 2.29	14.15 ± 0.47	89.33 ± 1.45
TIE 300 mg/kg + $K_2Cr_2O_7$	128.00 ± 1.73	12.92 ± 0.33^{tt}	89.50 ± 2.01
TIE 1000 mg/kg + $K_2Cr_2O_7$	124.00 ± 0.86^{t}	13.03 ± 0.50^{tt}	89.67 ± 1.20

All data are expressed as mean ± S.E.M. (n=6), *$p< 0.05$, **$p< 0.01$ when compared to control group (one way ANOVA followed by Sidak"s post hoc test). $^{t}p< 0.05$ and $^{tt}p< 0.01$ when compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak"s post hoc test).

Effect of TIE and $K_2Cr_2O_7$

3.4 EFFECT OF *TERMINALIA IVORENSIS EXTRACT* (TIE) ON POTASSIUM DICHROMATE ($K_2CR_2O_7$) – INDUCED OXIDATIVE STRESS

3.4.1 Effect of TIE and $K_2Cr_2O_7$ on reduced glutathione levels

Kidney GSH levels were significantly reduced ($p< 0.001$) in $K_2Cr_2O_7$ (20 mg/kg) and 100 mg/kg of extract groups when compared to the control group, whereas the extract at 300 and 1000 mg/kg significantly ($p< 0.001$) increased GSH levels as compared to the $K_2Cr_2O_7$ group. The extract at 300 and 1000 mg/kg however normalized the reduced glutathione levels when compared to the control group (figure 3.2).

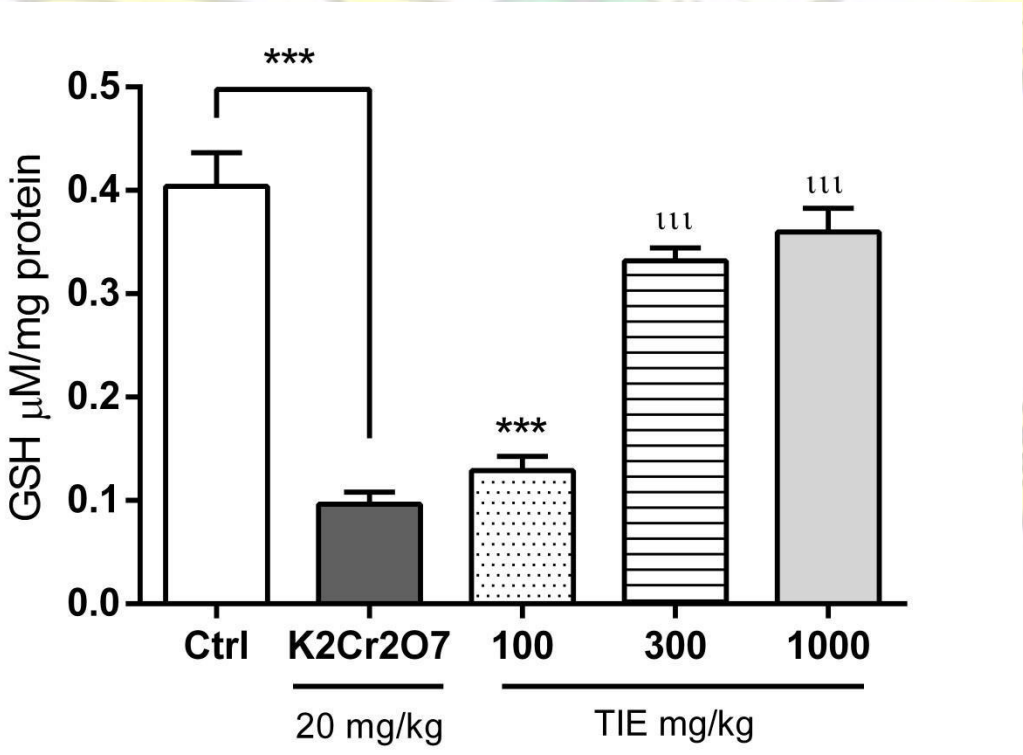


Figure 3.2: Effect of $K_2Cr_2O_7$ (20 mg/kg) and TIE (100, 300, 1000 mg/kg) on GSH levels. ***$p< 0.001$ when compared to control group (one way ANOVA followed by Sidak‟s post hoc

test). $^{ttt}p< 0.001$ when compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak‟s post hoc test).

3.4.2 on superoxide dismutase levels

A significant reduction ($p< 0.001$) in tissue SOD levels was found in the $K_2Cr_2O_7$ treated group as compared to the control. Pretreatment of rats with extract (TIE) significantly increased SOD levels at 100 mg/kg ($p< 0.01$) and $p< 0.001$ (300 and 1000 mg/kg of extract) when compared to the $K_2Cr_2O_7$ group (figure 3.3).

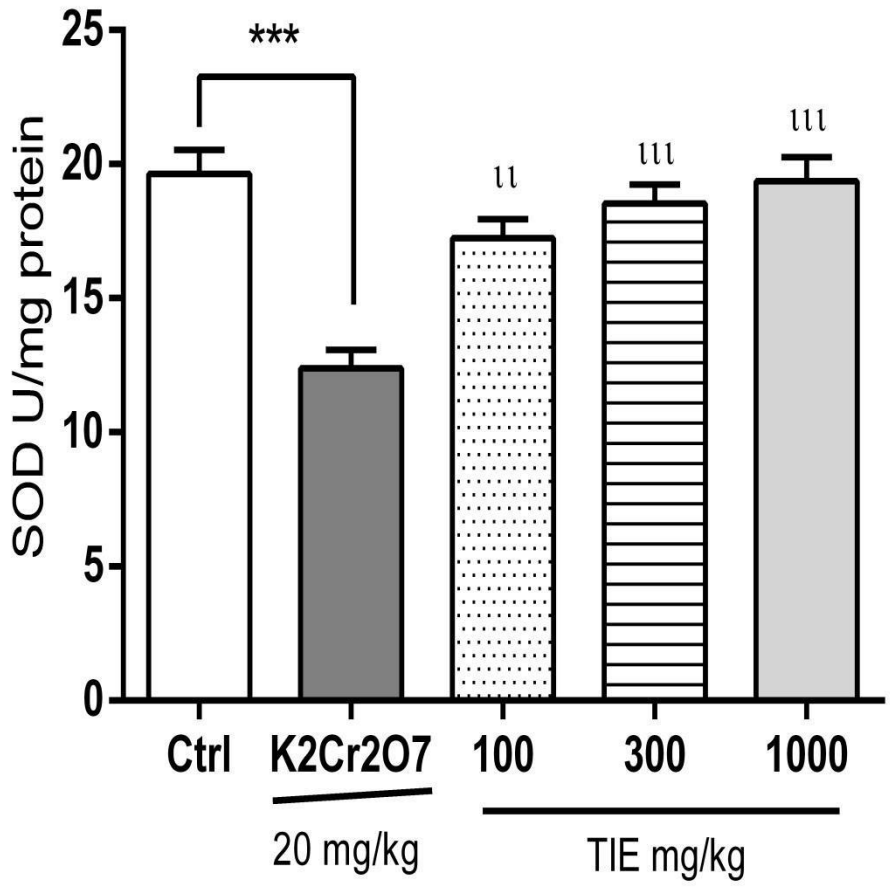


Figure 3.3: Effect of $K_2Cr_2O_7$ (20 mg/kg) and TIE (100, 300, 1000 mg/kg) on SOD levels.

Effect of TIE and $K_2Cr_2O_7$

***p< 0.001 when compared to control group (one way ANOVA followed by Sidak‟s post hoc test). ttp< 0.01 and tttp< 0.001 when compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak‟s post hoc test).

3.4.3 Effect of TIE and $K_2Cr_2O_7$ on catalase levels

Tissue catalase levels were significantly reduced ($p< 0.001$) in $K_2Cr_2O_7$ and 100 mg/kg of extract when compared to the control group. The extract (300 and 1000 mg/kg) however normalized the catalase levels as compared to the control group. However, the extract at 300 and 1000 mg/kg significantly ($p< 0.001$) increased catalase levels when compared to $K_2Cr_2O_7$ group (figure 3.4).

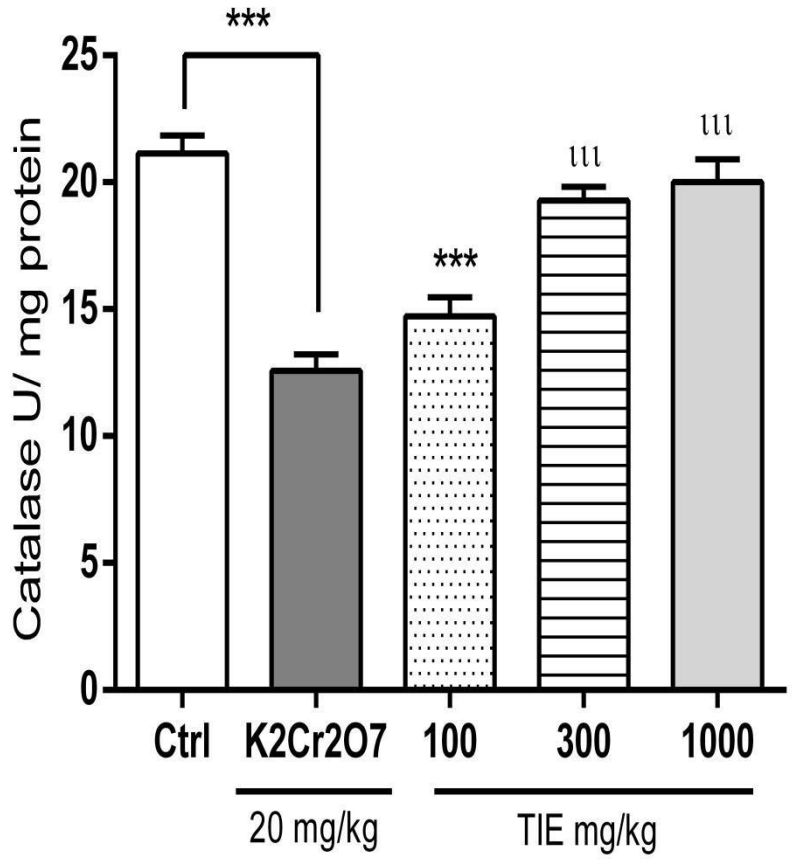


Figure 3.4: Effect of $K_2Cr_2O_7$ (20 mg/kg) and TIE (100, 300, 1000 mg/kg) on catalase levels. ***$p< 0.001$ as compared to the control group (one way ANOVA followed by Sidak‟s post hoc test), $^{ttt}p< 0.001$ when compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak‟s post hoc test).

3.4.4 Effect of TIE and $K_2Cr_2O_7$ on lipid peroxidation

Malondialdehyde levels after induction of oxidative stress with $K_2Cr_2O_7$ significantly increased in toxic group ($p< 0.001$) and 100 mg/kg of extract group ($p< 0.05$) when compared to the control group. MDA formation however significantly decreased in extract treated groups at 100 mg/kg ($p< 0.01$) and $p< 0.001$ (300 and 1000 mg/kg) when compared to $K_2Cr_2O_7$ group (figure 3.5).

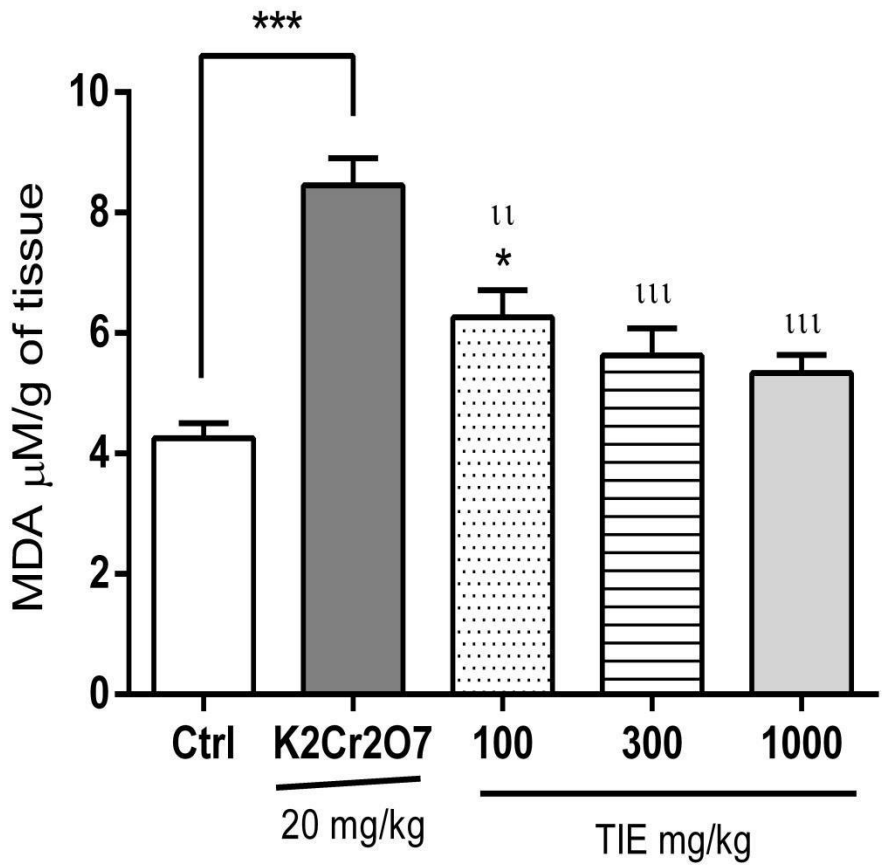


Figure 3.5: Effect of $K_2Cr_2O_7$ (20 mg/kg) and TIE (100, 300, 1000 mg/kg) on MDA levels. *$p< 0.05$ and ***$p< 0.001$ as compared to the control group (one way ANOVA followed by Sidak‟s post hoc test), ŧŧ$p< 0.01$ and ŧŧŧ$p< 0.001$ when compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak‟s post hoc test).

Effect of TIE and $K_2Cr_2O_7$

3.4.5 on myeloperoxidase (MPO) levels

Kidney myeloperoxidase formation significantly increased in $K_2Cr_2O_7$ group ($p< 0.001$) and 100 mg/kg of extract ($p< 0.05$) when compared to the control group, whereas extract pre – treated groups significantly ($p< 0.001$) decreased MPO formation when compared to the $K_2Cr_2O_7$ group (figure 3.6).

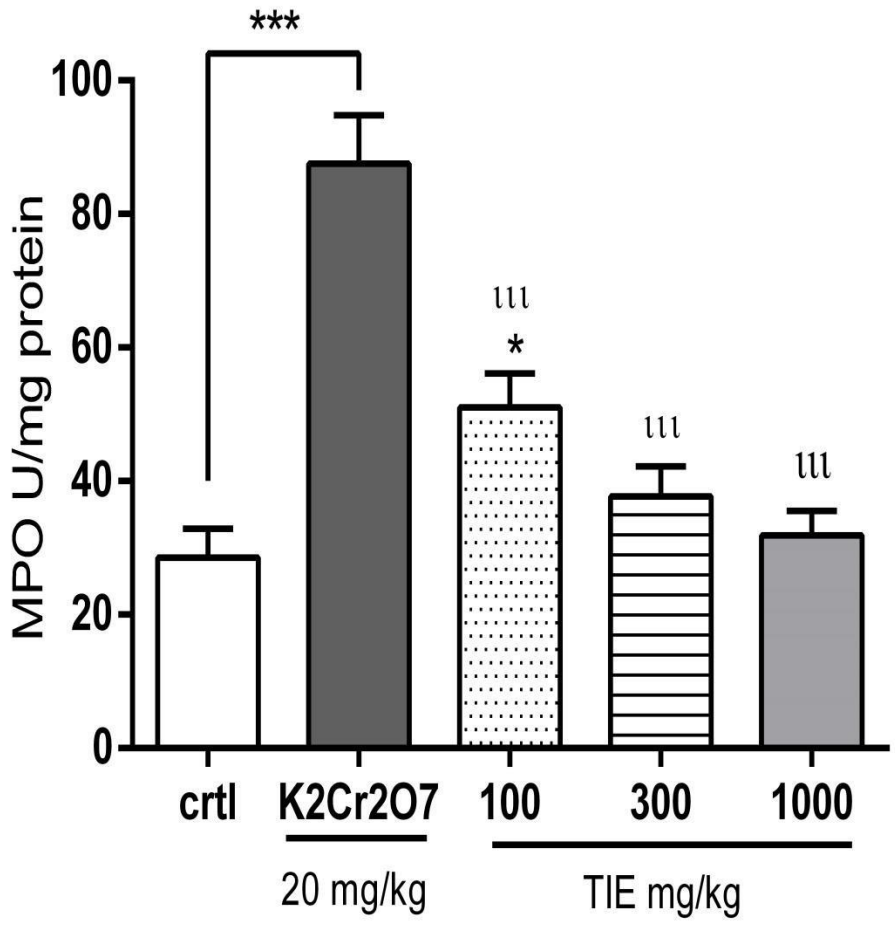


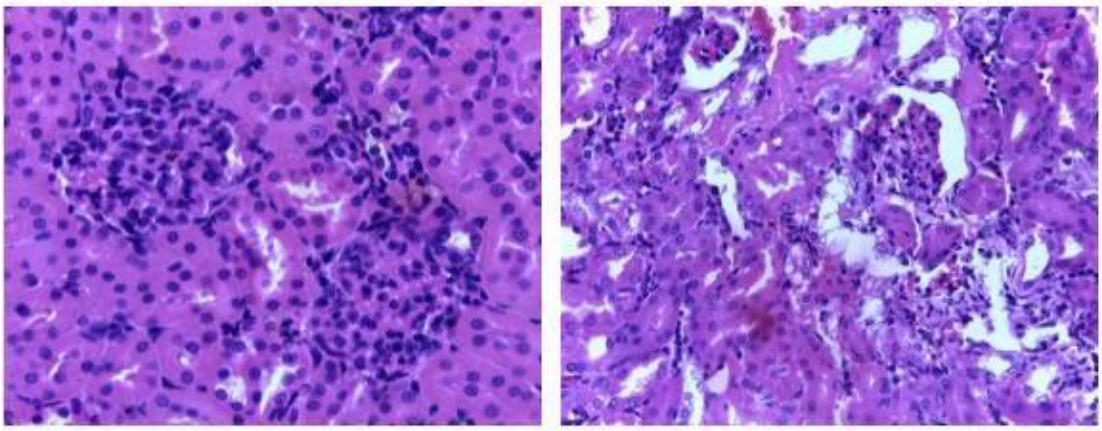
Figure 3.6: Effect of $K_2Cr_2O_7$ (20 mg/kg) and TIE (100, 300, 1000 mg/kg) on MPO levels.

Effect of TIE and $K_2Cr_2O_7$

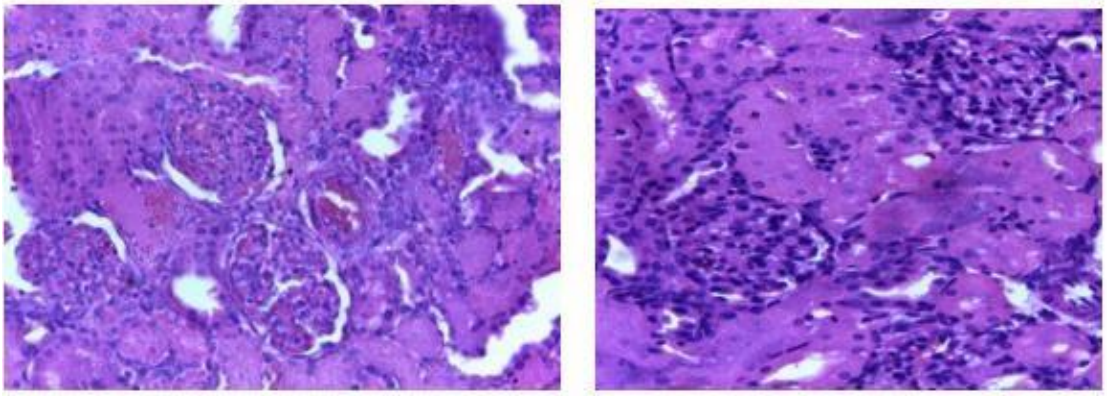
*p< 0.05 and ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak‟s post hoc test), ^{ttt}p< 0.001 when compared to $K_2Cr_2O_7$ group (one way ANOVA followed by Sidak‟s post hoc test).

3.4.6 Photomicrographs of kidneys showing the effect of TIE on $K_2Cr_2O_7$ – induced nephrotoxicity

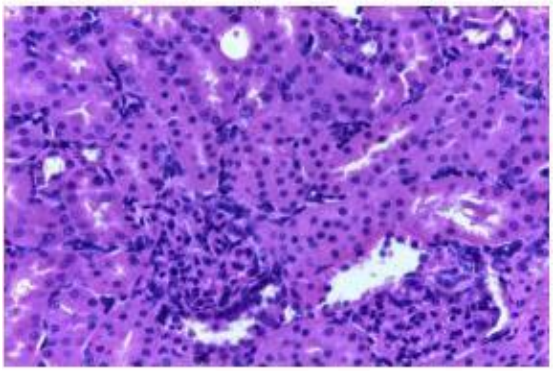
Examination of histopathological sections of kidneys indicated that the control group showed normal renal architecture characterized by normal glomerular and tubular histology. Potassium dichromate ($K_2Cr_2O_7$) treated group showed severe vacuolar degeneration, glomerular degeneration, tubular necrosis and tubular cast. However, pretreatment of rats with *Terminalia ivorensis* extract before administration of $K_2Cr_2O_7$ reduced the kidney injury. At 100 mg/kg of extract, there was a moderate kidney damage characterized by vacuolarization, moderate tubular casts, glomerular degeneration and necrosis. Whereas extract at 300 and 1000 mg/kg showed mild vacuolarization, glomerular degeneration, tubular casts and necrosis were observed (figure 3. 7).



(A) Control (B) $K_2Cr_2O_7$ (20 mg/kg)



(C) TIE (100 mg/kg) & $K_2Cr_2O_7$ (20 mg/kg) (D) TIE (300 mg/kg) & $K_2Cr_2O_7$ (20 mg/kg)



(E) TIE (1000 mg/kg) & $K_2Cr_2O_7$ (20 mg/kg)

Figure 3.7: Effect of $K_2Cr_2O_7$ (20 mg/kg) and TIE (100, 300, 1000 mg/kg bwt) on kidney histomorphology.

3.5 EFFECT OF *TERMINALIA IVORENSIS EXTRACT* (TIE) ON GENTAMYCIN – INDUCED NEPHROTOXICITY

3.5.1 Effect of gentamycin and TIE on urine volume

A significant increase urine volume was observed in extract pretreated groups at 300 ($p< 0.01$) and 1000 mg/kg ($p< 0.001$) as compared to the control. The extract (1000 mg/kg) also significantly ($p< 0.001$) elevated the urine output when compared to the gentamycin group (figure 3.8).

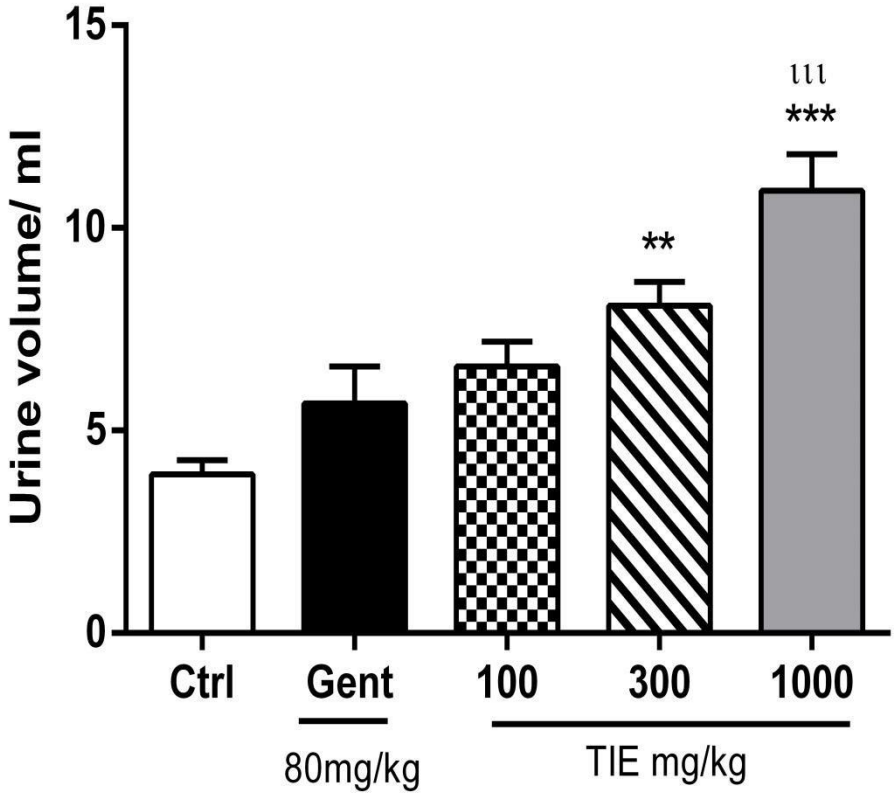


Figure 3.8: Effect of Gentamycin (80 mg/kg) and TIE (100, 300, 1000 mg/kg) on 24 – hour urine output. **$p< 0.01$, ***$p< 0.001$ as compared to the control group (one way ANOVA followed by Sidak‟s post hoc test) and $^{ttt}p< 0.001$ as compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

3.5.2 Effect of TIE and Gentamycin on body and kidney weights

Gentamycin (80 mg/kg) significantly ($p< 0.05$) decreased the percent change in body weight of rats as compared to the control. Whereas TIE (1000 mg/kg), significantly ($p< 0.05$) stabilized the weight loss as compared to the gentamycin group.

However, no observed difference in the weights of kidneys in the various treatment groups was seen when compared to the control (Table 3.6).

Table 3.6: Effect of TIE and gentamycin on kidney weight and % change in body weight

Treatment group	Kidney weight /g	% Change in body weight
Control: distilled 10 ml/kg	1.31 ± 0.09	-2.90 ± 0.64
Gent 80 mg/kg	1.51 ± 0.04	-12.45 ± 1.91*
TIE 100 mg/kg + Gent 80 mg/kg	1.27 ± 0.06	-9.82 ± 2.98
TIE 300 mg/kg + Gent 80 mg/kg	1.38 ± 0.04	-9.18 ± 2.35
TIE 1000 mg/kg + Gent 80 mg/kg	1.42 ± 0.12	-3.30 ± 0.64^{t}

All data are stated as mean ± S.E.M. (n=6), *$p< 0.05$ as compared to control group and $^{t}p< 0.05$ as compared to gentamycin group (one way ANOVA followed by Sidak"s post hoc test).

3.5.3 Effect of TIE and Gentamycin on Creatinine and urea

Creatinine levels were significantly elevated in 100 mg/kg of extract ($p< 0.05$) and gentamycin ($p< 0.001$) as compared to the control group, whereas significant decrease ($p< 0.001$) was observed in extract pre-treated groups when compared to the gentamycin group.

Again, gentamycin significantly ($p< 0.001$) increased the serum urea levels of rats when compared to the control. Whereas significantly diminished serum urea levels were observed at 300 ($p< 0.001$) and 1000 mg/kg of extract ($p< 0.01$) when compared to the gentamycin treated group (Table 3.7).

Table 3.7: Effect of TIE and Gentamycin on serum Creatinine and urea

Treatment group	Creatinine/ μmol/L	Urea/ mmol/L
Control: distilled 10 ml/kg	33.10 ± 1.77	11.66 ± 0.67
Gent 80 mg/kg	102.70±11.75***	22.42 ± 2.90***
TIE 100 mg/kg + Gent 80 mg/kg	59.03±2.78*^{ttt}	16.36 ± 0.99
TIE 300 mg/kg + Gent 80 mg/kg	47.83±3.51^{ttt}	12.86 ± 0.66^{ttt}
TIE 1000 mg/kg + Gent 80 mg/kg	53.48±5.74^{ttt}	13.21 ± 0.82^{tt}

All data are expressed as mean ± S.E.M. (n=6), *p< 0.05, **p< 0.01 and ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak‟s post hoc test). ^{tt}p< 0.01 and ^{ttt}p< 0.001 as compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

3.5.4 Effect of TIE and Gentamycin on serum sodium, potassium and chloride levels

Serum electrolyte levels were significantly (p< 0.001) elevated in gentamycin and 100 mg/kg of TIE treatment groups when compared to the control. At 300 mg/kg of TIE, a significant rise in potassium levels (p< 0.01) and chloride levels (p< 0.05) was observed when compared to the control group.

However, extract pre – treated rats at 1000 mg/kg normalized the electrolyte levels as compared to the control group. Again, extract pre – treated rats significantly (p< 0.001) lowered potassium levels whereas sodium levels significantly reduced at 300 (p< 0.01) and 1000 mg/kg (p< 0.001) when compared to the gentamycin group. Besides, significantly

diminished chloride levels were observed in extract pre – treated groups at 100 mg/kg ($p< 0.01$), 300 and 1000 mg/kg ($p< 0.001$) when compared to the toxic group (Table 3.8).

Table 3.8: Effect of TIE and Gentamycin on serum sodium, potassium and chloride levels

Treatment group	Sodium/ m/L	Potassium/ m/L	Chloride/ m/L
Control: distilled 10 ml/kg	115.3 ± 2.45	8.48 ± 0.46	78.33 ± 1.61
Gent 80 mg/kg	135.2 ± 2.80***	16.40 ± 0.50***	98.67 ± 1.71***
TIE 100 mg/kg + Gent 80 mg/kg	136.7±1.43***	13.10 ± 0.49***^{ttt}	90.67± 0.88***^{tt}
TIE 300 mg/kg + Gent 80 mg/kg	123.2 ±0.60^{tt}	11.00 ± 0.33**^{ttt}	84.83 ± 0.95*^{ttt}
TIE 1000 mg/kg + Gent 80 mg/kg	118.0 ± 1.77^{ttt}	9.53 ± 0.28^{ttt}	80.67 ± 0.76^{ttt}

All data are expressed as mean ± S.E.M. (n=6), *$p< 0.05$, **$p< 0.01$ and ***$p< 0.001$ when compared to the control group (one way ANOVA followed by Sidak‟s post hoc test). $^{tt}p< 0.01$ and $^{ttt}p< 0.001$ when compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

3.6 EFFECT OF *TERMINALIA IVORENSIS EXTRACT* (TIE) ON GENTAMYCIN – INDUCED OXIDATIVE STRESS

3.6.1 Effect of TIE and Gentamycin on reduced glutathione (GSH) levels

TIE (100 mg/kg, $p< 0.01$) and gentamycin (80 mg/kg, $p< 0.001$) significantly decreased tissue GSH levels of rats when compared to the control group. Whereas at 300 ($p< 0.01$) and 1000 mg/kg ($p< 0.001$), significantly raised GSH levels were observed when compared to the gentamycin group (figure 3.9).

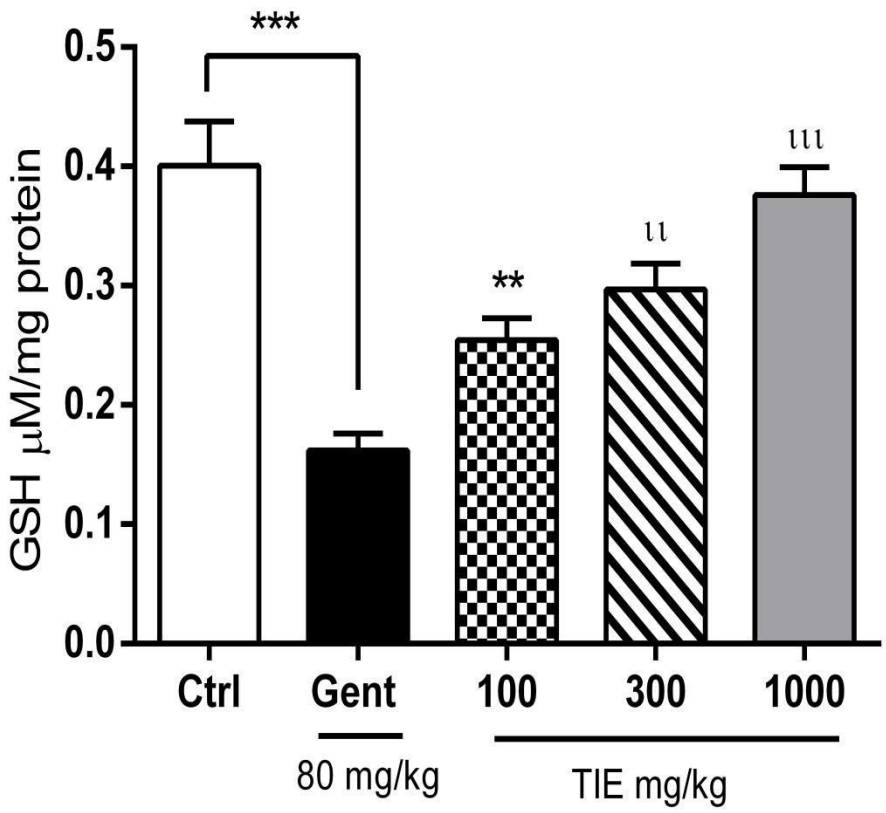


Figure 3.9: Effect of Gentamycin (80 mg/kg) and TIE (100, 300, 1000 mg/kg) on GSH levels. **p< 0.01 and ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak"s post hoc test). ŧŧp< 0.01 and ŧŧŧp< 0.001 when compared to gentamycin group (one way ANOVA followed by Sidak"s post hoc test).

3.6.2 Effect of TIE and Gentamycin on superoxide dismutase (SOD) levels

Induction of oxidative stress with gentamycin significantly decreased kidney superoxide dismutase levels in gentamycin treated group (p< 0.001) and 100 mg/kg of extract group (p< 0.05) when compared to the control. However, comparing SOD levels of toxic (gentamycin) group to the extract treated groups, showed significantly increased enzyme levels at 100 mg/kg (p< 0.01) and p< 0.001 for 300 and 1000 mg/kg of extract. The extract (300 and 1000 mg/kg) was however able to normalize SOD levels as compared to the control group (figure 3.10).

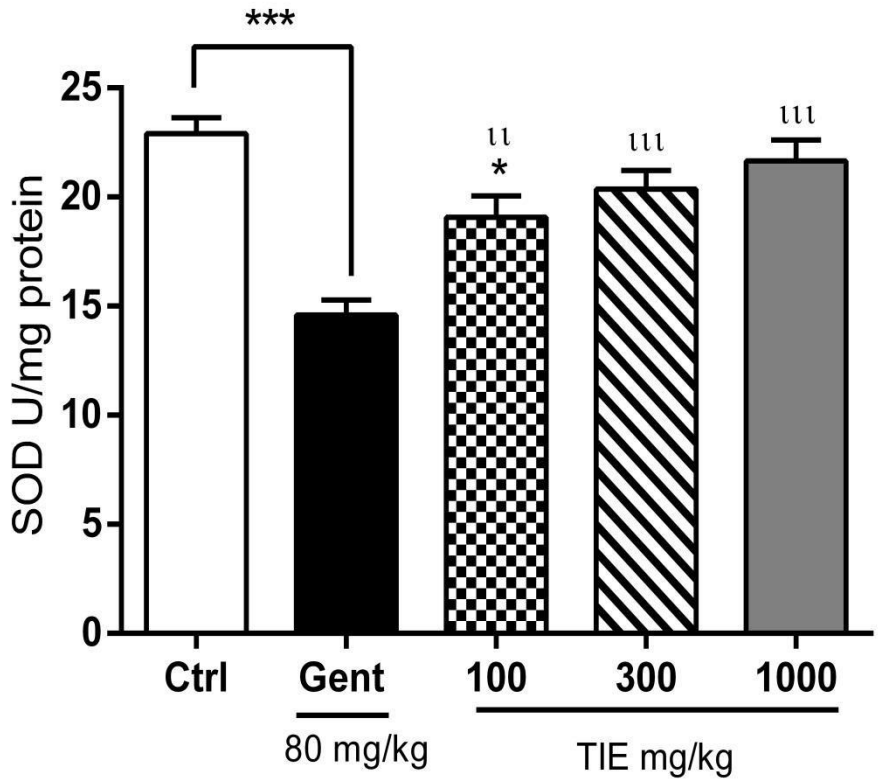


Figure 3.10: Effect of Gentamycin (80 mg/kg) and TIE (100, 300, 1000 mg/kg) on SOD levels. *p< 0.05 and ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak‟s post hoc test). ^{tt}p< 0.01 and ^{ttt}p< 0.001 when compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

3.6.3 Effect of TIE and Gentamycin on catalase levels

A significantly (p< 0.001) reduced catalase levels were observed in gentamycin (80 mg/kg) and 100 mg/kg of extract as compared to the control group, whereas the extract at 300 and 1000 mg/kg significantly (p< 0.001) elevated catalase levels as compared to the gentamycin group. The extract at 300 and 1000 mg/kg however normalized the catalase levels as compared to the control group (figure 3.11).

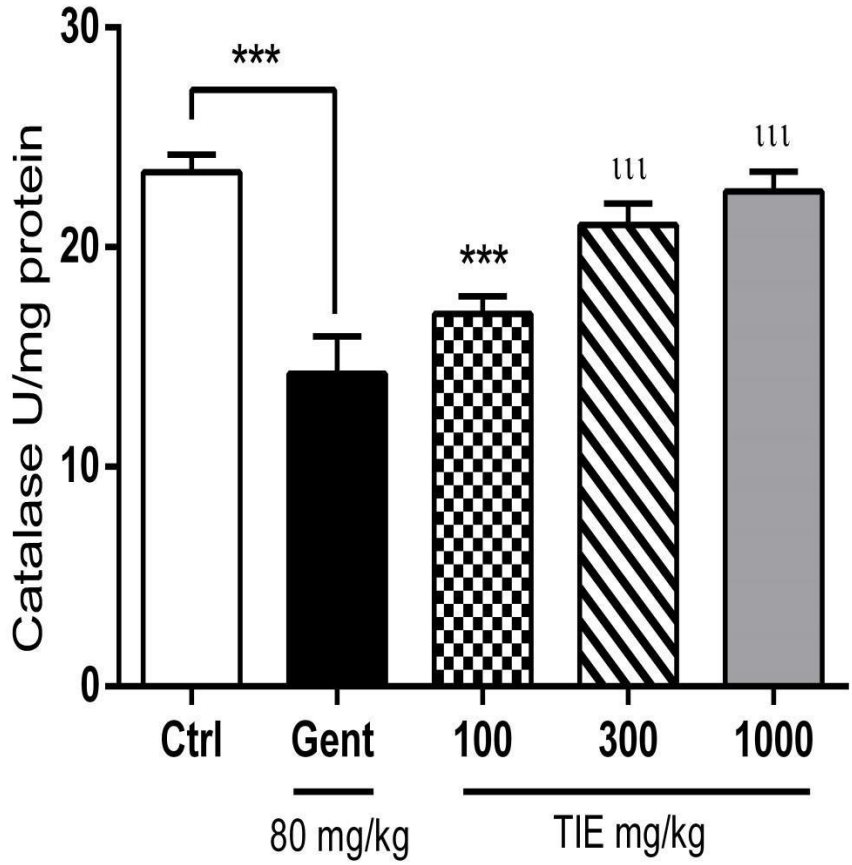


Figure 3.11: Effect of Gentamycin (80 mg/kg) and TIE (100, 300, 1000 mg/kg) on catalase levels. ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak"s post hoc test) and ^{ttt}p< 0.001 when compared to gentamycin group (one way ANOVA followed by Sidak"s post hoc test).

3.6.4 Effect of TIE and Gentamycin on lipid peroxidation

Lipid peroxidation was assessed in terms of the amount of malondialdehyde (MDA) produced. Induction of oxidative stress with gentamycin significantly increased the amount of MDA produced in gentamycin treated group (p< 0.001) and 100 mg/kg of extract group (p< 0.05) when compared to the control group. MDA formation was however significantly decreased in extract at 100 (p< 0.05), 300 (p< 0.01) and 1000 mg/kg (p< 0.001) as compared to gentamycin group (figure 3.12).

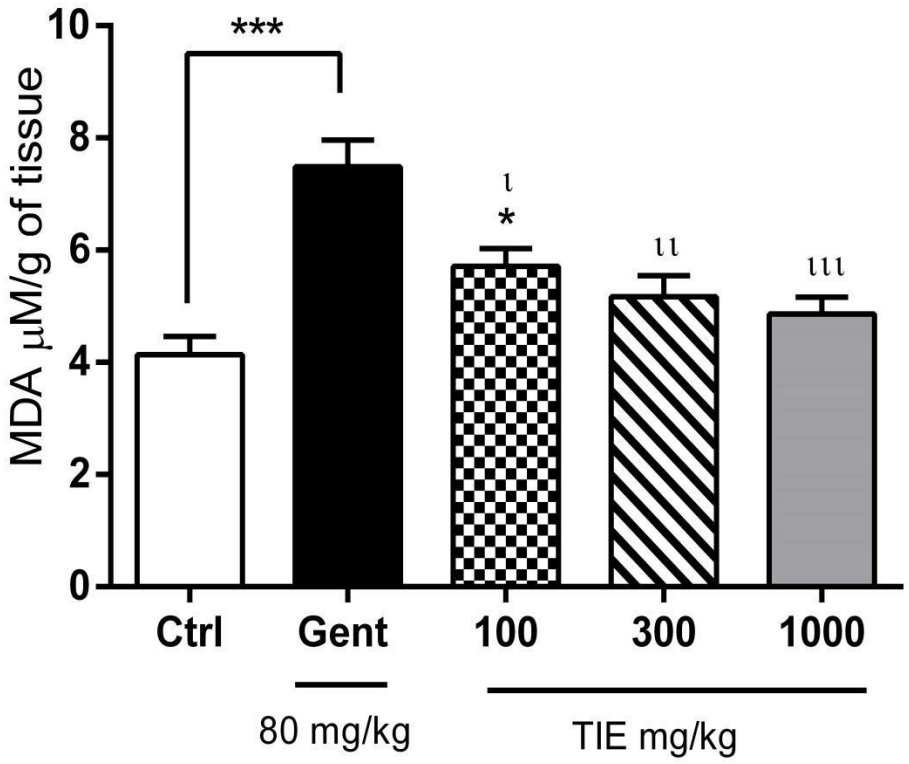


Figure 3.12: Effect of Gentamycin (80 mg/kg) and TIE (100, 300, 1000 mg/kg) on MDA levels. *p< 0.05 and ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak"s post hoc test). ιp< 0.05, ιιp< 0.01 and ιιιp< 0.001 when compared to gentamycin group (one way ANOVA followed by Sidak"s post hoc test).

3.6.5 Effect of TIE and Gentamycin on myeloperoxidase (MPO) levels

A significantly increased (p< 0.001) tissue MPO levels was observed in gentamycin (80 mg/kg) and TIE (100 mg/kg) treated groups as compared to the control group. The extract pretreated rats at 300 and 100 mg/kg significantly (p< 0.01) decreased MPO formation when compared to the gentamycin group (figure 3.13).

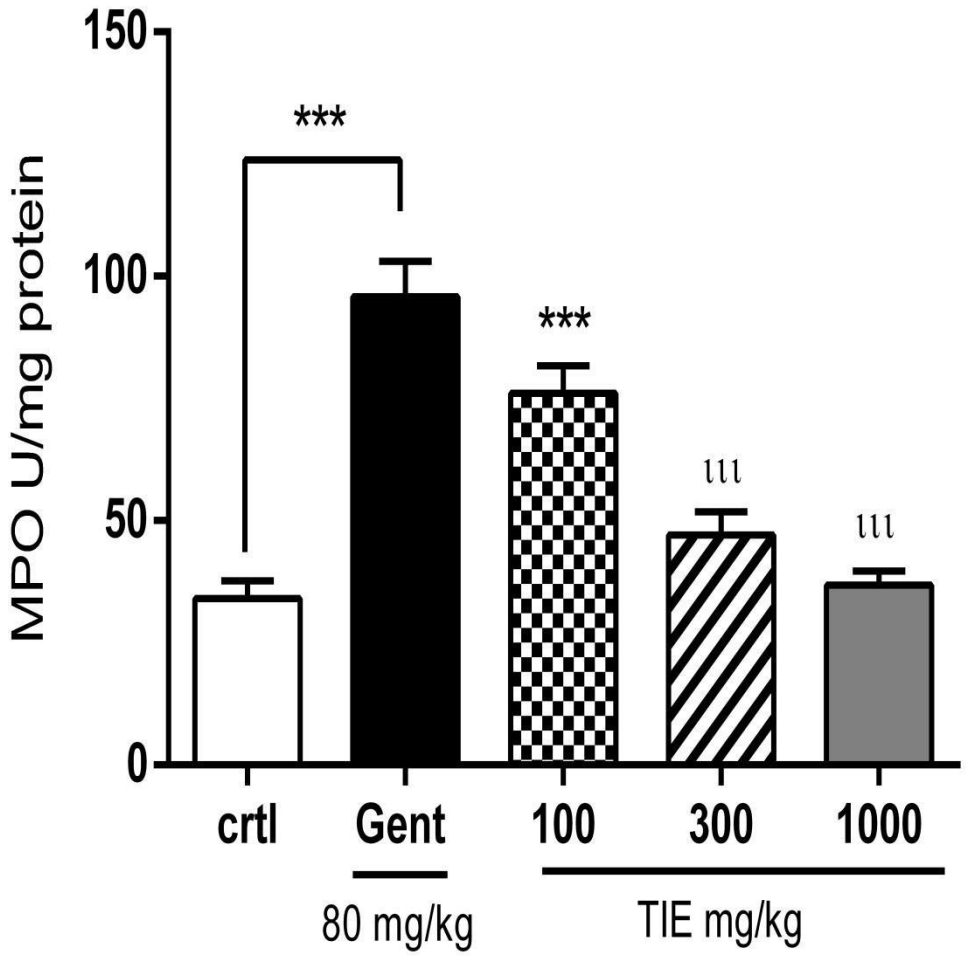
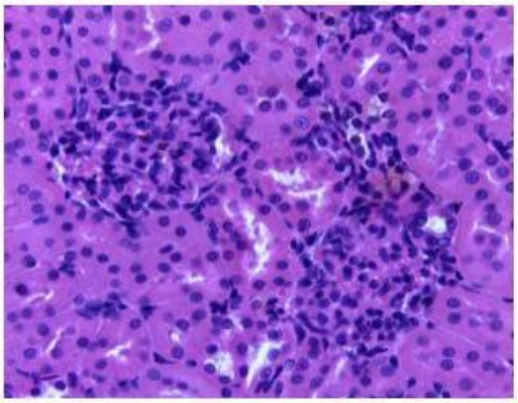


Figure 3.13: Effect of Gentamycin (80 mg/kg) and TIE (100, 300, 1000 mg/kg) on MPO levels. ***p< 0.001 as compared to the control group (one way ANOVA followed by Sidak‟s post hoc test) and ^{ttt}p< 0.001 when compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

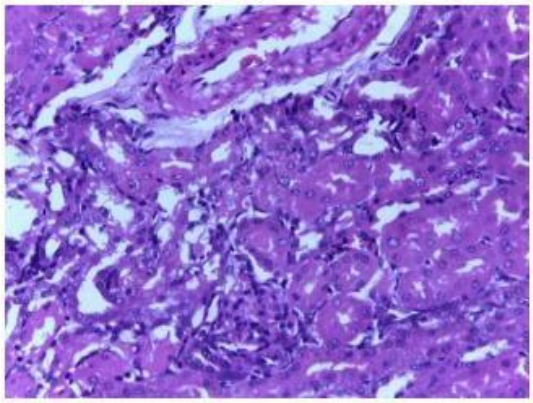
3.6.6 Photomicrographs of kidneys showing the effect of TIE on gentamycin – induced nephrotoxicity

Assessment of histomorphology of kidney sections showed normal glomerular and tubular kidneys in the medullar and cortical regions of kidneys in the control group. Gentamycin was shown to cause severe glomerular degeneration, vacuolization, tubular casts and necrosis. In contrast, pretreatment of rats with extract at 100 mg/kg showed a moderate reduction of kidney

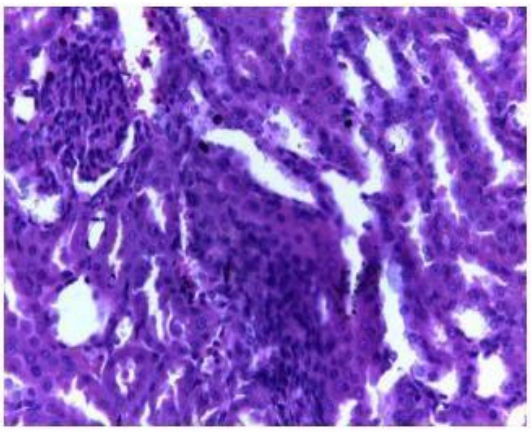
histology features of injury. Again, pretreatment of rats with extract at 300 and 1000 mg/kg was linked with more decrease in renal injury similar to control group (figure 3.14).



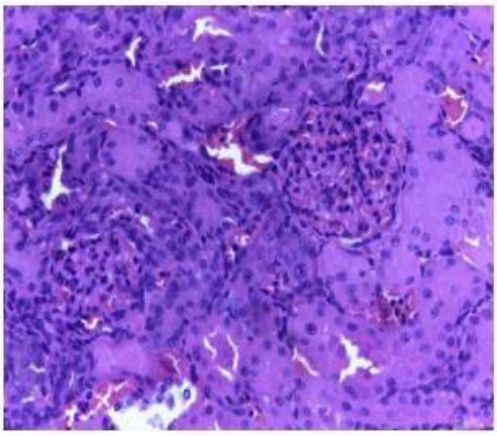
(A) Control



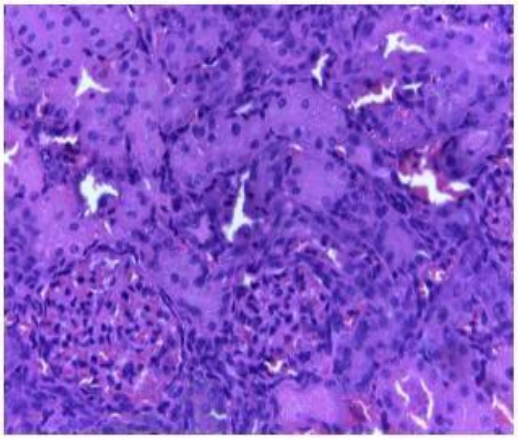
(B) Gent (80 mg/kg)



(C) TIE (100 mg/kg) & gent (80 mg/kg)



(D) TIE (300 mg/kg) & gent (80 mg/kg)



(E) TIE (1000 mg/kg) & gent (80 mg/kg)

Figure 3.14: Effect of gentamycin 80 mg/kg and TIE (100, 300, 1000 mg/kg bwt) on kidney histomorphology

3.7 EFFECT OF *TERMINALIA IVORENSIS* AGAINST GENTAMYCIN – INDUCED

HEPATOTOXICITY

3.7.1 Effect of TIE and Gentamycin on body and liver weights

There no significant difference between the body and liver weights in the various treatment groups as compared to the control group and also no observed significant difference was seen when the extract treated groups were compared to the gentamycin group (Table 3.9). **Table 3.9**: Effect of TIE and Gentamycin on liver weights and % change in body weight

Treatment group	Liver weight /g	% Change in body weight
Control: distilled 10 ml/kg	6.23 ± 0.22	2.40 ± 0.67
Gent 80 mg/kg	5.71 ± 0.35	1.95 ± 1.58
TIE 100 mg/kg + Gent 80 mg/kg	5.24 ± 0.30	2.12 ± 2.42
TIE 300 mg/kg + Gent 80 mg/kg	5.60 ± 0.07	2.26 ± 1.61
TIE 1000 mg/kg + Gent 80 mg/kg	5.37 ± 0.29	2.38 ± 1.28

All data are expressed as mean ± S.E.M. (n=6).

3.7.2 Effect of TIE and Gentamycin on AST, ALT and GGT

Significant ($p < 0.05$) elevation in AST levels were observed in gentamycin treated group when compared to the control group whereas 1000 mg/kg of extract significantly ($p < 0.05$) diminished AST levels when compared to the gentamycin group.

ALT levels were observed to be significantly ($p< 0.01$) high in gentamycin group as compared to the control. The extract however, significantly reduced ALT levels at 300 ($p< 0.01$) and 1000 mg/kg ($p< 0.05$) as compared to the gentamycin group.

Gentamycin again, significantly ($p< 0.01$) increased GGT levels when compared to the control whilst 1000 mg/kg of extract significantly ($p< 0.05$) reduced GGT levels when compared to the gentamycin group.

Table 3.10: Effect of TIE and Gentamycin on AST, ALT and GGT levels

Treatment group	AST / U/L	ALT/ U/L	GGT / U/L
Control: distilled 10 ml/kg	298.8 ± 4.9	116.2 ± 13.1	1.6 ± 0.12
Gent 80 mg/kg	985.5 ± 270.5*	218.8 ± 29.6**	4.6 ± 0.77**
TIE 100 mg/kg + Gent 80 mg/kg	709.0 ± 145.5	142.8 ± 15.6	3.8 ± 0.73
TIE 300 mg/kg + Gent 80 mg/kg	447.4 ± 38.9	122.2 ± 15.9^{t}	2.8 ± 0.46
TIE 1000 mg/kg + Gent 80 mg/kg	361.2 ± 26.8^{t}	126.2 ± 4.3^{t}	2.0 ± 0.17^{t}

All data are expressed as mean ± S.E.M. (n=6), *$p< 0.05$ and **$p< 0.01$ as compared to control group. $^{t}p< 0.05$ and $^{tt}p< 0.01$ as compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

3.7.3 Effect of TIE and Gentamycin on ALP, total bilirubin, and total protein levels

Induction of liver damage with gentamycin, significantly ($p< 0.01$) increased ALP levels when gentamycin group was compared to the control whilst 1000 mg/kg of extract significantly ($p< 0.05$) reduced ALP levels when compared to the gentamycin group.

Total bilirubin levels were observed to be significantly ($p< 0.01$) elevated in gentamycin group as compared to the control. The extract however, significantly reduced total bilirubin levels at 300 ($p< 0.05$) and 1000 mg/kg ($p< 0.01$) as compared to the gentamycin group.

Assessment of total protein levels showed a significant decrease in gentamycin group ($p< 0.001$) and $p< 0.05$ (100 and 300 mg/kg of extract) as compared to the control group. Extract at 1000 mg/kg however significantly ($p< 0.01$) raised total protein levels as compared to the gentamycin group.

Table 3.11: Effect of TIE and Gentamycin on ALP, total bilirubin, and total protein levels

Treatment group	ALP / U/L	Total bilirubin/ µmol/L	Total protein / g/L
Control: distilled 10 ml/kg	176.9 ± 23.7	4.2 ± 0.49	71.0 ± 0.81
Gent 80 mg/kg	296.7 ± 15.5**	6.3 ± 0.49**	56.3 ± 2.63***
TIE 100 mg/kg + Gent 80 mg/kg	228.4 ± 13.2	4.9 ± 0.16	61.6 ± 2.65*
TIE 300 mg/kg + Gent 80 mg/kg	224.9 ± 32.2	4.6 ± 0.19^{t}	61.2 ± 1.14*
TIE 1000 mg/kg + Gent 80 mg/kg	189.6 ± 14.3^{t}	4.2 ± 0.25^{tt}	66.3 ± 1.34^{tt}

All data are expressed as mean ± S.E.M. (n=6), *$p< 0.05$, **$p< 0.01$ and ***$p< 0.001$ as compared to control group. $^{t}p< 0.05$ and $^{tt}p< 0.01$ as compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

3.7.4 Effect of TIE and Gentamycin on globulin and albumin levels

Globulin levels were significantly low ($p< 0.01$) in gentamycin group when compared to the control whereas extract at 300 and 1000 mg/kg significantly increased ($p< 0.05$) globulin levels as compared to the gentamycin group.

A significant decrease in albumin levels were observed in gentamycin ($p< 0.001$) and $p< 0.05$ (100 and 300 mg/kg of extract) as compared to the control group. Extract at 1000 mg/kg however significantly elevated ($p< 0.05$) albumin levels as compared to the gentamycin group.

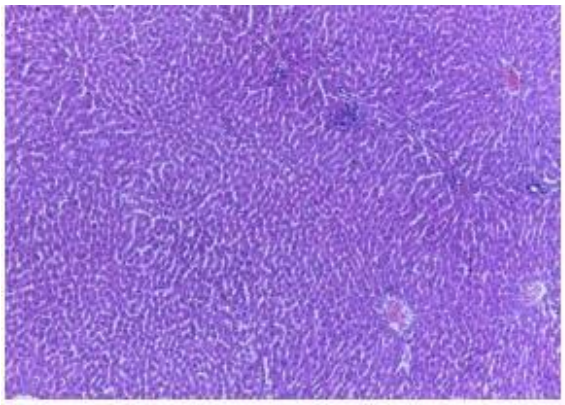
Table 3.12: Effect of TIE and Gentamycin on globulin and albumin levels

Treatment group	Globulin / g/L	Albumin / g/L
Control: distilled 10 ml/kg	25.5 ± 0.62	44.3 ± 0.94
Gent 80 mg/kg	19.8 ± 0.82**	37.7 ± 0.71***
TIE 100 mg/kg + Gent 80 mg/kg	22.6 ± 0.86	39.7 ± 0.54*
TIE 300 mg/kg + Gent 80 mg/kg	24.5 ± 1.40^{t}	40.3 ± 1.45*
TIE 1000 mg/kg + Gent 80 mg/kg	24.1 ± 0.88^{t}	41.8 ± 0.47^{t}

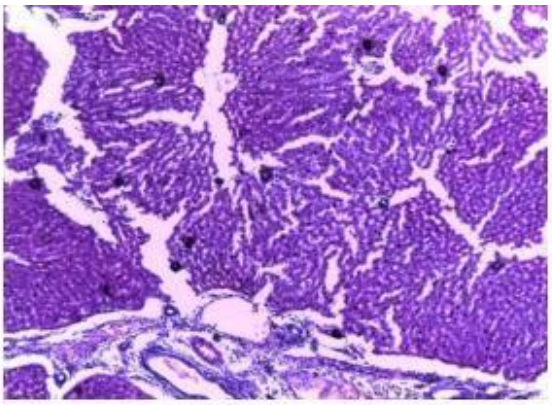
All data are expressed as mean ± S.E.M. (n=6), *p< 0.05, **p< 0.01 and ***p< 0.001 as compared to control group. ^{t}p< 0.05 as compared to gentamycin group (one way ANOVA followed by Sidak‟s post hoc test).

3.7.5 Photomicrographs of liver sections

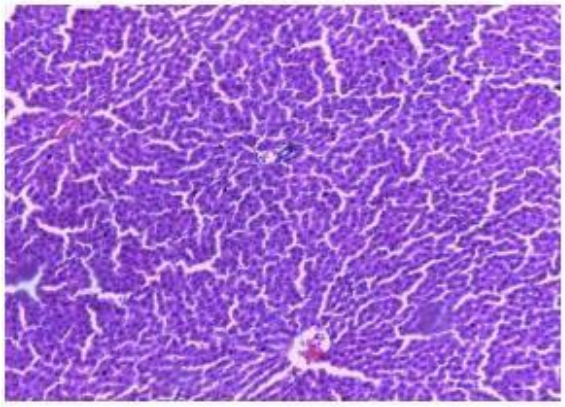
Assessment of liver histology showed normal liver histology in control group. However, the gentamycin group showed congested hepatic sinusoids and blood vessels, apoptotic cells, severe vacuolization and necrosis. Extract pretreated groups, showed normal liver architecture with no visible lesions similar to the control group.



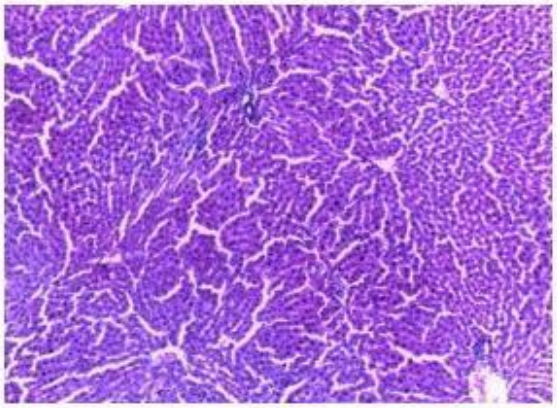
(A) Control



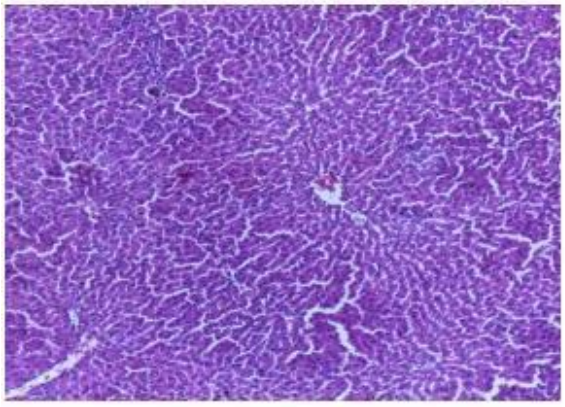
(B) Gent (80 mg/kg)



(C) 100 mg/kg & gent (80mg/kg)



(D) 300 mg/kg & gent (80 mg/kg)



(E) 1000 mg/kg & gent (80 mg/kg)

Figure 3.15: Effect of gentamycin 80 mg/kg and TIE (100, 300, 1000 mg/kg) on liver histology.

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

4.1 DISCUSSION

Medicinal plants serve a crucial purpose as the first line management of various disease conditions due to their low cost, easy accessibility and minimal side effects (Gopalakrihnan and Dhanapal, 2014; Annan *et al.*, 2012). In spite of the wide usage of herbal plants for the cure of many disease conditions, the safety or potential toxicity of many of these plants have not been scientifically investigated (Ansah *et al.*, 2009).

Among the plants used by herbalists include *Terminalia ivorensis* which is used for ulcers, wounds, diuresis, general body pains, malaria, hemorrhoids and yellow fever (Ouattara *et al.*, 2013; Akinyemi *et al.*, 2006; Etukudo, 2003; Burkill, 1985). It is also used as timber, anticough, antihypertensive, anti-hyperglycemic, anti-parasitic, anti-inflammatory and anti-arthritis as well as for skin and oral infections (Johnny *et al.*, 2014; Agbedahunsi *et al.*, 2006; Iwu and Anyanwu, 1982).

However, very little is known about its toxicity, nephroprotective and hepatoprotective effects. Thus, the present study was planned to assess the nephroprotective effect of *Terminalia ivorensis* ethanolic stem bark extract against potassium dichromate and gentamycin induced nephrotoxicity in Sprague Dawley rats.

Secondary metabolites from plants serve an essential purpose in the defense of plants against microorganisms, preys, stress and interspecies protection (Cowan, 1999). The occurrence of a particular secondary metabolite in a plant may be useful in deducing the potential effect of the plant when taken as food or drug (Nale *et al.*, 2012). The availability of saponins, flavonoids, tannins, terpenoids, steroids and alkaloids are attributed to the anti-diabetic, anti-parasitic, antidiarrhea, anti-inflammatory, anti-ulcer and anti-malarial properties of herbal preparations (Annan *et al.*, 2012; Iwu and Anyanwu, 1982) whereas cardiac glycosides account for the cardiac and neurotropic activities of these herbal preparations (Li and Ohizumi, 2004).

Phytochemical screening of the ethanolic stem bark extract of *Terminalia ivorensis* indicated the existence of saponins, alkaloids, flavonoids, tannins, terpenoids, steroids, cardiac glycosides and anthraquinones which agreed with the works of Coulibaly *et al.,* (2014), Johnny *et al.,* (2014); and Annan *et al.,* (2012).

Acute toxicity denotes the effects of a single dose or multiple doses of a substance during a 24 - hour period (Sathya *et al.,* 2012). It is one of the initial steps in the assessment of the toxic properties of compounds (Bhardwaj and Gupta, 2012; Akhila *et al.,* 2007). This toxicity studies is carried out for short periods (usually 24 hours) to establish the toxicity, safety and efficacy profile of a new product. After a single administered dose, samples are usually observed for a few days or 2 – weeks (Pacific BioLabs, 2009). LD_{50} is the index for studying acute toxicity (Abdullah, 2011). No death was recorded when the extract (TIE) was administered to rats, hence, the median lethal dose (LD_{50}) was estimated to be above 5000 mg/kg body weight. Clarke *et al.* (1975) reported that any substance with LD_{50} beyond 1000 mg/kg should be regarded nontoxic or have low toxicity when orally administered.

Nephrotoxicity is an adverse effect of chemotherapy (Chatterjee *et al.,* 2012). Drug or chemical induced nephrotoxicity depends on their build-up in renal cortex and binding of the substance to kidney structures (Noorani *et al.,* 2010).

For example, chromium is an essential trace element in mammalian tissues that is involved in the metabolism of protein, glucose and lipid (Wang *et al.,* 2006; Mertz, 1993). It usually accumulates in the renal tubules and is urinary excreted (Klaassen, 2008; Pedraza-Chaverri *et al.,* 2008). Potassium dichromate, the hexavalent form of chromium, is a strong oxidizing agent shown to cause oxidative stress and mutagenesis (Pedraza-Chaverri *et al.,* 2008; Mertz, 1969).

It is a common element of organic matter and its levels in human depend on the diet taken, age and syndromes of the endocrine system (Mertz, 1993). It is a commonly used manufacturing

chemical (e.g. in alloy, paints, steel, textile manufacture, cooling systems, wood preservation, cast irons, photography, leather tanning) and is known to be carcinogenic and cytotoxic in nature (Aslam *et al.*, 2013; Galvao and Corey, 1987).

Then again, gentamycin is a commonly used aminoglycoside used in the cure of gram-negative infections (Noorani *et al.*, 2010). Due to its potential nephrotoxic and ototoxic properties, the use has been constrained (Luft and Kleit, 1974). This aminoglycoside is highly charged and water soluble at physiological pH and thus, unable to pass through biological membranes (Alfthan *et al.*, 1973). It therefore, usually collects in the renal proximal tubules and promotes hydrogen peroxide production by the mitochondria (Noorani *et al.*, 2010). The hydrogen peroxide produced causes the release of iron from mitochondria membranes which complexes with gentamycin to hasten the production of reactive oxygen products (Afeefa *et al.*, 2012).

The nephrotoxic effects of gentamycin have been strongly linked to its preferential accumulation in renal cortex (Luft and Kleit, 1974). The occurrence of nephrotoxicity induced by aminoglycoside has increased by 20 % over the past decade (Leehey *et al.*, 1993). This antibiotic has been shown to dose dependently induce nephrotoxicity when administered for more than 7-10 days (Noorani *et al.*, 2010; Geevasinger *et al.*, 2006). Gentamycin at 80 mg/kg body weight administered intraperitoneally for two weeks in rats is shown to result in significant kidney dysfunction and destruction (Chatterjee *et al.*, 2012; Qadir *et al.* 2011). Beside, exposure of an organism to gentamycin causes a build-up of oxidative stress in the system (Chatterjee *et al.*, 2012; Gowrisri *et al.*, 2012) which is evidenced by diminished levels of GSH, SOD and catalase as well as increase in lipid peroxidation and cellular damage (Gowrisri *et al.*, 2012).

In this study, potassium dichromate (after administering 20 mg/kg) and gentamycin (80 mg/kg administered for 14 days), induced renal dysfunction which was exhibited by significant increase in serum urea, creatinine, electrolytes and decreased body weight with various histological destruction.

This was in agreement with earlier studies by Aslam *et al.,* (2013) which showed elevated levels of serum markers (urea and creatinine) during $K_2Cr_2O_7$ induced nephrotoxicity. PedrazaChaverri *et al.,* (2008) also reported that subcutaneous treatment of rats with $K_2Cr_2O_7$ induced nephrotoxicity evidenced by elevated serum urea, creatinine and structural injury.

In other studies in agreement with the present findings, Vidya *et al.,* (2013), Chatterjee *et al.,* (2012) and Qadir *et al.* (2011) reported that after induction of nephrotoxicity with gentamycin, there were significantly elevated levels of serum creatinine, urea and damage of renal structures in the toxic group.

Pretreatment of rats with *Terminalia ivorensis* ethanolic stem bark extract at 300 and 1000 mg/kg before induction of nephrotoxicity, significantly increased the urine output and body weight (at 1000 mg/kg) when compared to the toxic group. Due to its ability to increase urine output, *Terminalia ivorensis* could prove useful in the management of diuresis. Hence, the extract could be used as a diuretic. Induction of renal injury with potassium dichromate and gentamycin steered the build-up of metabolic waste like ammonia and urea which instigated generalized fatigue and reduced intake of food leading to weight loss (Rajitha *et al.,* 2013).

These results agreed with previously documented work by Rajitha *et al.,* (2013) and Chatterjee *et al.,* (2012) who reported decrease in body weight in the toxic group. Qadir *et al.* (2011) and Vidya *et al.,* (2013) however, reported no observed difference in body weights in comparing the controls and extract treated groups, which differed from my findings.

In this study however, no observed difference was seen in the kidney weights between the control, extract treated and toxic groups. The elevated levels of serum markers were significantly reduced in extract pretreated groups (300 and 1000 mg/kg) when compared to the toxic groups. The reduction in the renal markers (creatinine and urea) and serum electrolytes (sodium, potassium and chloride) demonstrated that the extract is probably minimizing cell

damage caused by $K_2Cr_2O_7$ and gentamycin thereby improving renal function. Chatterjee *et al.*, (2012) however reported no significant difference in serum electrolyte levels in the toxic and extract treated groups which disagreed with my findings.

In numerous animal models, oxidative stress has been strongly linked to nephrotoxicity. Superoxide dismutase, catalase and GSH are the primary defense systems against reactive oxygen species. These antioxidants get oxidized when they neutralize a free radical and thus, needs to be frequently replenished to prevent toxicity as result of the accumulation of free radicals (Aslam *et al.*, 2013; Adejuwon *et al.*, 2014).

Superoxide dismutase is involved in converting superoxide radicals to oxygen and hydrogen peroxide (Abreu and Cabelli, 2009). It is the first line of defense against reactive oxygen species (Abreu and Cabelli, 2009; Larry and Garry, 1979). Catalase decomposes hydrogen peroxide to water and oxygen (Sivanandham, 2011; Weydert and Cullen, 2009). GSH is a soluble tripeptide that acts as an antioxidant and a redox buffer (Main *et al.*, 2012; Townsend *et al*., 2003).

During nephrotoxicity, there is a build – up of reactive species, which results in an oxidative degradation of lipids and other lipid – containing structures (Girotti, 1998) in a process termed lipid peroxidation. Lipid peroxidation was quantified as amount of malondialdehyde (MDA) formed. A high level of MDA indicates high level of cell membrane degradation due to lipid peroxidation (Adejuwon *et al.*, 2014; Girotti, 1998). Myeloperoxidase (MPO) is also involved in the development of tissue damage and increased levels of free MPO are observed in tissues sites with inflammation (Arnhold, 2004). MPO converts hydrogen peroxide and chloride ions into hypochlorous acid (HOCl) and other highly reactive products (Amy *et al.*, 2014; Metzler *et al.*, 2011).

In this study, nephrotoxicity induced by potassium dichromate and gentamycin was evidenced by diminished SOD, GSH and catalase levels as well as elevated MDA, MPO and tissue

damage. Extract pretreated animals showed significantly enhanced reduced glutathione (GSH), superoxide dismutase (SOD), and catalase levels as compared to the toxic groups. Whereas, the formation of myeloperoxidase and lipid peroxidation product (MDA) were also observed to be significantly reduced when compared to the toxic groups. The activity shown by *Terminalia ivorensis* ethanolic stem bark extract may be as a result of its ability to induce antioxidants in the system. This agreed with earlier study done by Aslam *et al.,* (2013) who reported reduced oxidative stress markers (GSH, catalase and superoxide dismutase) and high lipid peroxidation during $K_2Cr_2O_7$ induced nephrotoxicity.

Kidney sections of the control rodents showed normal architecture of tubules while the toxic ($K_2Cr_2O_7$ and gentamycin) showed severe glomerular degeneration, vacuolization, tubular casts and necrosis. However, extract pretreated rats at 100 mg/kg showed a moderate reduction of kidney histology features of injury. Again, pretreatment of rats with extract at 300 and 1000 mg/kg was linked with more decrease in renal injury similar to control group. Pretreatment of rats with extract offered a protective effect against renal damage caused by $K_2Cr_2O_7$ and gentamycin.

Once more, gentamycin has been shown to induce hepatotoxicity secondary to nephrotoxicity and ototoxicity. It induces hepatic injury by promoting the generation of free radicals which attack and destroy hepatocytes (Al – Kenannay *et al.,* 2012; Noorani *et al.,* 2010). Drug – induced hepatotoxicity is characterized by elevated levels of ALT, AST, ALP, GGT and bilirubin as well as destruction of tissue histology (Adejuwon *et al.,* 2014; Sivakrishnan and Kottaimuthu, 2013; Kurtovic and Riordan, 2003).

High levels of these enzymes are consequences of hepatocytes destruction and increase in cellular permeability (Sivakrishnan and Kottaimuthu, 2013), whereas increased amount of

bilirubin is indicative of loss of functional efficiency of hepatic cells in binding, conjugating and excreting bilirubin (Singh *et al.,* 2005; Saroswat *et al.,* 1993).

In this study, gentamycin has been shown to significantly increase the levels of liver biomarkers (ALT, AST, ALP, GGT and bilirubin) and decrease levels of total proteins, globulin and albumin as well as cause severe liver destruction. However, co – administration of extract and gentamycin significantly normalized the levels of these markers and restored liver structure.

This work agreed with earlier works of Al – Kenannay *et al.,* (2012) and Noorani *et al.,* (2010) who also reported that gentamycin induces hepatotoxicity evidenced by elevated levels of liver markers.

Therefore, ethanolic stem bark extract of *Terminalia ivorensis* was able significantly reduce the damage caused by gentamycin probably through a protective effect against cellular destruction and restoring hepatocytes integrity.

4.2 CONCLUSIONS

Terminalia ivorensis has a nephroprotective effect against potassium dichromate and gentamycin induced nephrotoxicity evidenced by alterations in renal markers and reduced toxicity to the kidney.

The possible mechanism of nephroprotection of *T. ivorensis* could probably be through the induction of the activities of endogenous antioxidants in the system.

It also has hepatoprotective effect on gentamycin induced hepatotoxicity by enhancing liver function and restoring liver cells integrity.

4.3 RECOMMENDATIONS

Terminalia ivorensis is a potential diuretic, thus, patients on diuretics should take caution when taking this plant extract, to avoid potentiated effect of diuretics. It is also recommended that isolation and characterization of the active compound of *T. ivorensis* should be carried out.

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