TITLE PAGE

EVALUATION OF THE PROTECTIVE EFFECT OF AQUEOUS AND ETHANOL FRUIT EXTRACTS OF *Phoenix dactylifera L* ON MERCURY - INDUCED LIVER AND KIDNEY DAMAGE IN WISTAR RATS

BY

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DEPARTMENT OF HUMAN ANATOMY,

FACULTY OF MEDICINE,

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JUNE, 2017.

DECLARATION

I, Musa Garba ABUBAKAR, hereby declare that the work in the thesisentitled “Evaluation of the protective effect of aqueous and ethanolic fruit extracts of *Phoenix dactyliferaL* on mercury - induced liver and kidney damage in Wistar rats” has been carried out by me in the Department of Human Anatomy.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at this or any other institution.

_____ _____

Musa Garba ABUBAKAR Date

CERTIFICATION

This thesis entitled "EVALUATION OF THE PROTECTIVE EFFECT OF AQUEOUS AND ETHANOL FRUIT EXTRACTS OF *Phoenix dactylifera L* ON MERCURY - INDUCED LIVER AND KIDNEY DAMAGE IN WISTAR RATS" by Musa Garba ABUBAKAR meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the newest addition to our family, Fareeda Ikram.

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All praise and glory be to God Almighty, the Lord of the world for it is by His infinite mercies, blessings and guidance that I have come this far against all odds.

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ABSTRACT

Mercury is a highly toxic metal that exerts its adverse effects on the health of humans and animals worldwide. The present study was aimed at evaluating the protective effects of aqueous and ethanol extracts of *Phoenix dactylifera L.* on mercury induced liver and kidney damage in Wistar rats. A total of forty five (45) male Wistar rats of (80 – 125g) were randomly divided into nine groups (I-IX) of five (5) rats each. Group 1 served as the Control and was administered distilled water (0.5 ml), Group II was administered mercury chloride ($HgCl_2$) only at 5 mg/kg body weight (bwt); Group III was pretreated with silymarin at 100 mg/kgbwt then followed by $HgCl_2$ at5 mg/kgbwt; Groups IV and V were pretreated with aqueous fruit extract of *Phoenix dactylifera* (AFPD) at 500 mg/kgbwt and 1000 mg/kgbwt, respectively, followed by $HgCl_2$ at 5 mg/kgbwt; Groups VI and VII were pretreated withethanol fruit extract of *Phoenix dactylifera*(EFPD) at 500mg/kgbwt and 1000 mg/kgbwt, respectively, followed by $HgCl_2$ at 5 mg/kgbwt, while Groups VIII and IX were pretreated with AFPD and EFPD only at 1000 mg/kgbwt, respectively. After two weeks of oral administration, the animals were sacrificed using cervical dislocation method and blood samples were collected through the jugular vein for biochemical studies (liver serum enzymes: Aspartate transaminase (AST), Alaninetransaminase (ALT) and Alkaline phosphatase (ALP); and oxidative stress markers, Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxide (GPx). Tissue samples of the liver and kidney were collected and processed for light microscopy using routineHaematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS) and Gordon and Sweet (GS) stains. The results of the present study showed that toxicity and oxidative stress were induced by the significant increased levels of AST, ALP and MDA ($p<0.05$)

and decreased levels of SOD, CAT and GPx when compared to the Control. Histological and histochemical changes in the liver and kidney revealed severe degenerative changes, such as hepatocellular vacuolation, sinusoidal dilatation, pyknotic nuclei and cytoplasmic vacuolation in rats exposed to $HgCl_2$ when compared to the Control. The administration of extracts of AFPD and EFPDpreserved liver serum enzymes, kidney electrolytes and antioxidant enzyme activities to levels similar to that of the Control and provided protection against mercury-induced changes in the liver and kidney of Wistar rats. The protective activities of the extracts of *Phoenix dactylifera* could be attributed to the antioxidant properties such as flavonoids. Thus, these extracts could be potential candidates for use in the management and treatment of mercury-related liver and kidney diseases in our localities.

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LIST OF ABBREVIATIONS

AFPD	Aqueousfruitextractof *Phoenix dactylifera*
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances Disease Registry
BDH	British Drug Houses
bwt	Body weight
CARB	California Air Resources Board
CAT	Catalase
Cu	Copper
DNA	Deoxyribonucleic acid
EFPD	Ethanol fruit extract of *Phoenix dactylifera*
EPA	Environmental Protection Agency
GPx	Glutathione peroxidase
GSH	Glutathione
H&E	Haematoxylin and Eosin
Hg^0	Elemental mercury
$HgCl_2$	Mercuric chloride
kg	Kilogram
LSD	Least significant difference
MDA	Malondialdehyde
MeHg	Methylmercury
mg	Milligram

ml	Milliliters
P. dactylifera	*Phoenix dactylifera*
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBARS	Thiobarbituricacid-reactive-substances
UNEP	United Nations Environment Programme
WHO	World Health Organisation
Zn	Zinc

CHAPTER ONE

1.0 Introduction

1.1 Background of the Study

Mercury is a widespread environmental and industrial pollutant that exerts toxic effect on a variety of vital organs; it induces severe alterations in the tissues (Lund *et al.*, 1993; Mahboob *et al.*, 2001; Sener *et al.*, 2007; Wadaan, 2009; Xu *et al.*, 2012). Mercury will cause severe disruption of any tissue with which it comes into contact in sufficient concentration, but the two main effects of mercury poisoning are neurological and renal disturbances. The former is characteristic of poisoning by methyl- and ethyl mercury (II) salts while the latter is characteristic of poisoning by inorganic mercury such as mercuric chloride in which liver and renal damage is of relative significance.

Inorganic mercury compounds are rapidly accumulated in the kidney, the main target organ for these compounds. The biological half-time is very long, probably years, in both animals and humans. Mercury salts are excreted via the kidney, liver, intestinal mucosa, sweat glands, salivary glands and milk; the most important routes are via the urine and faeces (IPCS, 2003). Mercury is absorbed through inhalation, ingestion and dermal contact, its absorption is related to the type of mercury compound and the duration of exposure (Collasiol *et al.*, 2004). In general, mercurial are attracted to sulfhydryl radicals in the body and are bound to proteins, membranes and enzymes, altering their normal functioning (Yee *et al.*, 1996; WHO, 2003). Mercury poisoning has been reported in humans following exposure to metallic mercury and its organic and inorganic derivatives (Kelly *et al.*, 2006). Inorganic mercury accumulates primarily in the kidneys, liver, spleen,

bone marrow, intestine, skin and respiratory mucosa (EUESA, 2007). Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into blood stream (Wadaan, 2009). High exposures to inorganic mercury may result in damage to the gastrointestinal tract, the nervous system, and the kidneys (US EPA, 2010). Both inorganic and organic mercury compounds are absorbed through the gastrointestinal tract and affect other systems via this route. However, organic mercury compounds are more readily absorbed via ingestion than inorganic mercury compounds. Symptoms of high exposures to inorganic mercury include: skin rashes and dermatitis; mood swings; memory loss; mental disturbances; and muscle weakness (US EPA, 2010).

Despite massive efforts in search of new drugs that could counteract mercurial toxicity, there is no effective treatment available that can completely abolish its toxic effects. In mercurial poisoning, supportive care is given when necessary to maintain vital functions. In addition, the use of chelating agents assists the body's ability to eliminate mercury from the tissues (Carvalho *et al.,* 2007). However, these drugs appear to be of limited use, because of their adverse side effects (Tchounwou *et al.,* 2003). Since these medicines have certain serious side effects, there is an urgent need to systematically evaluate plants for their activities. Such research could also lead to new discovery or advance the use of indigenous herbal medicines for orthodox treatment (Uma *et al.,* 2012).

Date palm (*Phoenix dactylifera*) fruits, an important component of diet in the arid and semiarid regions of the world (Biglari *et al.,* 2008) is a good source of energy, vitamins

such as vitamin A, vitamin B6 (pyridoxine), vitamin K; and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium (Abdel-Hafez *et al.,* 1980; Usama *et al.,* 2009). Dates palm friuts contain vitamins and are widely used in traditional medicine for the treatment of various disorders, e.g., memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders (Nadkarni, 1976). It is also used in the treatment of sore throat, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication (Barh and Mazumdar, 2008; Al-daihan and Bhat, 2012).

The cultural use of medicinal plants is wide spread in Africa (Ashafa and Olunu, 2011). The World Health Organization (WHO) estimates that up to 80% of the world's population relies on traditional medicinal system for some aspect of primary health care (Farnsworth *et al.*, 1985). *Phoenix dactylifera L.* (date palm) is known to be one of the oldest cultivated trees in the world (Dowson, 1982; Abdullah, 2008). It has been an important crop in arid and semiarid regions of the world. It has always played an important part in the economic and social lives of the people of these regions. The fruit of the date palm is well known as a staple food. It is composed of a fleshy pericarp and mesocarp which encloses a single pit or stone (seed), therefore, it is a drupe. Date palms have been cultivated in the Middle East over at least 6000 years ago (Copley *et al.,* 2001). *Phoenix dactylifera Linn* is well known in northern Nigeria (with almost desert-like climate); the states in this axis are Jigawa, Borno, Kebbi, Yobe, Sokoto, Katsina and Zamfara) (Okere *et al.,*, 2010).

Many pharmacological studies have been conducted on *Phoenix dactylifera* and it has been demonstrated to have anti-ulcer activity; anti-cancer activity; anti-diarrhoeal activity; hepatoprotective activity; anti-mutagenic activity; anti-inflammatory activity; *in vitro* antiviral activity; effect on reproductive system; anti-hyperlipidemic activity; nephroprotective activity and antioxidant activity (Vyawahare *et al.*, 2009). Several researchers have also documented the antioxidant property of *Phoenix dactylifera* (Mohamed and Al-Okbi, 2004; Allaith and Abdul, 2005; Al-Qarawi *et al.*, 2008). Researchers pay more attention to the protective or therepeutic role of antioxidants, mainly phenolic compounds, found in natural plants as they may exhibit better antioxidant activity than already established antioxidant drugs such as vitamins C and E (Vinson *et al.*,, 1998; Haslam, 2006).

1.2 Statement of the Research Problem

Mercury is present in various environmental media and food, and has caused a variety of documented, significant adverse impacts on human health and wildlife throughout the world. Some studies have focused their efforts on the protective effects of natural compounds in various nephropathological and hepatopathological conditions (Esmaeili *et al.*, 2012; Uma *et al.*, 2012). However, the nephroprotective and hepatoprotective potential of higher plants as sources for new drugs is still largely unexplored. There is paucity of literature on the potential beneficial effects of aqueous and ethanolic fruit extracts of *P. dactylifera* against the nephrotoxicity and hepatotoxicity induced by mercuric chloride in Wistar rats.

1.3 Significance of the Study

The results of the present study could lead to the discovery of *P. dactylifera* as a more effective, affordable and safe agent in combating the problems of liver and kidney related disorders caused by mercury poisoning in developing countries which may be used as an alternative to currently used synthetic drugs, often accompanied by side effects.

1.4 Hypothesis

Aqueous and ethanol fruit extracts of *Phoenix dactylifera* has protective effect on mercury-induced alterations in the liver and kidney of Wistar rats.

1.5 Aim and Objectives of the Study

1.5.1 Aim

The aim of the study was to evaluate the protective effects of aqueous and ethanolic fruit extracts of *Phoenix dactylifera L.* on mercury-induced nephrotoxicity and hepatotoxicity in Wistar rats.

1.5.2 Objectives

The objectives of the present study were:

1. To investigate the protective effects of aqueous and ethanolic extracts of *P. dactylifera* on mercury-induced hepatotoxicity and nephrotoxicity in Wistar rats using routine histological and histochemical techniques.
2. To investigate the protective effect of aqueous and ethanolic extracts of *P. dactylifera* on mercury-induced hepatotoxicity and nephrotoxicity in Wistar rats using biochemical analyses for liver serum markers, kidney electrolytes, lipid

peroxide and antioxidant enzyme activity namely Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GSHPx).

3. To investigate the protective effects of aqueous and ethanolic extracts of *P. dactylifera* on mercury-induced hepatotoxicity and nephrotoxicity in Wistar rats using an established antioxidant (Silymarin) as a standard.

CHAPTER TWO

2.0 Literature Review

2.1 Mercury

Heavy metals as a source of environmental pollution have contributed immensely to severe illnesses and deaths in lower animals and human beings for centuries. A heavy metal is any relatively dense metal or metalloid noted for its potential toxicity, especially in environmental context (Srivastava *et al.,* 2010). Pollution by these elements could be by air, water or land depending on the activities resulting to their (heavy metals) exposure such as mining, industrial and agricultural activities, medical treatment, scientific researches among others. Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they can neither be degraded nor destroyed, and become concentrated as a result of human activities. However, these elements lack in abundance but not in significance (Chen and Chen, 2001). Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water (Adedokun *et al.,* 1989; Galadima *et al.,* 2011). Although, some elements regarded as heavy metals are essential, in small quantities, for human health, others such as cadmium, lead, arsenic and mercury are featured in the World Health Organization's list of ten (10) chemicals of major public concern (WHO, 2015).

Mercury is a chemical element with symbol '*Hg'* and atomic number 80. The symbol *Hg* that mercury is known by comes from its Greek name "hydragranum" which means liquidsilver to reflect its shiny surface. It is commonly known as quicksilver because of its

mobility. The element was named after the Roman god Mercury, known for his speed and mobility. Mercury is the only metallic element that is liquid at standard conditions for temperature and pressure (Davis, 2009). Mercury occurs in deposits throughout the world mostly as cinnabar (mercuric sulfide). The red pigment vermilion, a pure form of mercuric sulfide, is mostly obtained by reaction of mercury produced by reduction from cinnabar with sulfur. Mercury poisoning can result from exposure to water-soluble forms of mercury such as mercuric chloride or methylmercury, inhalation of mercury vapor, or eating seafood contaminated with mercury (Hammond, 2005). Mercury is used in thermometers, barometers, manometers, sphygmomanometers, float valves, mercury switches, mercury relays, fluorescent lamps and other devices, though concerns about the element's toxicity have led to mercury thermometers and sphygmomanometers being largely phased out in clinical environments in favor of alternatives, such as alcohol- or galinstan-filled glass thermometers and thermistor- or infrared-based electronic instruments. Likewise, mechanical pressure gauges and electronic strain gauge sensors have replaced mercury sphygmomanometers. However, mercury remains in use in scientific research applications and in amalgam for dental restoration in some localities (Hammond, 2005).

Mercury exists in three predominant forms: elemental (Hg^0), organic (such as, methylmercury –MeHg), and inorganic (mercuric chloride – $HgCl_2$) mercury (Clarkson and Magos, 2006). Each form has its own effects, routes of absorption and tissue specificity. Organic mercury is the most deadly of the mercury compounds, probably due to its ability to penetrate cells (Abdel-Salam *et al.*, 2010).

The studies about mercury (Hg) toxicity became ubiquitous and diversified since the outbreak of environmental catastrophes such as those in Minamata in the 1950s and Niigata in the 1960s (Takeuchi *et al.,* 1979; Eto, 2000). The burden of liver disease has increased in the world, and some cases are believed to be due to nonalcoholic fatty liver disease (NAFLD) caused by accumulation of heavy metal such as Hg (Cave *et al.,* 2007). The liver is the filter in our body. The liver cleanses our blood and eliminates unwanted substances like chemical toxins or debris from our blood stream. When we overload ourselves with toxins such as alcohol or are exposed to increased levels of heavy metals, at a certain point the protective detoxifying capacity of the liver runs out. When healthy liver cells dysfunction or die, fat cells are deposited and replace them. A "fatty" liver is the result. Serum alanine aminotransferase (ALT) is the most specific of the routinely used biomarkers for hepatocellular liver injury and disease in clinical medicine (Green and Flamm, 2002). Currently, there is no serologic biomarker to confirm the diagnosis of NAFLD, but ALT elevation (above normal laboratory reference ranges) is the most common laboratory manifestation of NAFLD (Clark, 2006).

Mercury can cause sodium retention and other electrolyte imbalances, which contribute to impaired kidney function (Goyer *et al.,* 1977). The inorganic form of mercury is toxic to kidney tissues due to the co-enzyme inhibition via high affinity to various microsome and mitochondria enzymes (Goyer *et al.,* 2001). Also, renal glomerular and tubular lesions have been associated with mercury toxicity in controlled laboratory studies (Afonne *et al.,* 2002), in humans, wildlife and in domestic mammals. Besides having detrimental effect on

other organ systems it is also capable of inducing nephrotoxicity (WHO, 1991) as kidneys form major route of its excretion (Noorafshan, 1998). Autopsy studies of mercury exposed humans showed that among soft tissues, liver is the largest repository followed by kidney (Sharma *et al.,* 2010). It is suggested that the mechanism involved in mercury induced toxicity is the metal induced reactive oxygen species (Ercal *et al.,* 2001) which are the initiators of peroxidative damage to the membranes (Adegbesan and Adenuga 2007; Sandhir *et al.,* 1994) and cause impaired membrane function, impaired structural integrity and inactivation of number of membrane bound enzymes (Sidhu *et al.,* 2004). Mecury-induced impairment of proximal tubular function manifests as enzymuria (ATSDR, 1999).

The major sources of mercury load in humans are food contamination, drug and vaccine preservatives, dental amalgams, or occupational exposure (Clarkson, 2002). Both acute and chronic exposure to mercury is also known to cause a variety of neurological or psychiatric disorders (Choi *et al.,* 1988; Meyer-Baron *et al.,* 2002; Clarkson *et al.,* 2003; Mutter *et al.,* 2005).

Ever since the commencement of the industrial age, the presence of mercury in environmental media and food especially fish and other aquatic animals, all over the world has increased to levels which negatively affects humans and wildlife. Even regions with no significant mercury releases in the past, such as the Arctic region, are adversely affected due to the transcontinental and global transport of mercury (UNEP, 2002).

2.1.1 Chemistry

The chemical and physical properties of mercury are shown in **Table 2.1**.

Table 2.1: Chemical and physical properties of mercury

CHEMICAL PROPERTIES	
Atomic number	80
Standard atomic weight	200.592(3)
Element category	transition metal
Group, **block**	group 12, d-block
Period	period 6
Electron configuration	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$
Per shell	2, 8, 18, 32, 18, 2
ATOMIC PROPERTIES	
Oxidation states	4, 2 (mercuric), 1 (mercurous) (a mildly basic oxide)
PHYSICAL PROPERTIES	
Phase	liquid
Melting point	234.3210 K (−38.8290 °C, −37.8922 °F)
Boiling point	629.88 K (356.73 °C, 674.11 °F)

(Lide, 2005).

2.1.2 History of Mercury

From ancient times, the history of mercury has been connected with that of medicine and chemistry. Mercury therefore contributes to the history of science throughout time (Norn *et al.,* 2008). Mercury was found in Egyptian tombs that date from 1500 BC (FGC, 2004). In China and Tibet, mercury use was thought to prolong life, heal fractures, and maintain good health, although it is now known that exposure to mercury vapor leads to serious adverse health effects (CEHS, 2012). The first emperor of China, Qín Shǐ Huáng Dì was allegedly buried in a tomb that contained rivers of flowing mercury on a model of the land he ruled. The emperor was reportedly killed by drinking a mixture of mercury and powdered jade formulated by his alchemists causing liver failure, mercury poisoning, and brain death. This mixture was intended to give him eternal life (Wright, 2001; Shihuang, 2003).

The ancient Greeks used mercury in ointments; the ancient Egyptians and the Romans used it in cosmetics which sometimes deformed the face (Colter, 2014). In Lamanai, once a major city of the Maya civilization, a pool of mercury was found under a marker in a Mesoamerican ballcourt (Pendergast, 1982). By 500 BC mercury was used to make amalgams with other metals (Hesse, 2007).

The largest natural source of mercury is cinnabar, it's only known ore, and the richest deposits are found in Spain and Italy. This reddish mineral containing mercury and sulfur has been used as a pigment since prehistoric times. Cinnabar dating from 500 BC has been identified at a Mayan site in Peru, and prehistoric skulls painted with cinnabar have been found in Italy (Sloane, 2012).

Alchemists thought of mercury as the first Matter from which all metals were formed. They believed that different metals could be produced by varying the quality and quantity of sulfur contained within the mercury. The purest of these was gold, and mercury was called for in attempts at the transmutation of base (or impure) metals into gold, which was the goal of many alchemists (Stillman, 2003).

Historically, preparations of mercury have been used in the treatment of diseases. Mercury (I) chloride (also known as calomel or mercurous chloride) has been used in traditional medicine as a diuretic, topical disinfectant, and laxative. Mercury (II) chloride (also known as mercuric chloride or corrosive sublimate) was once used to treat syphilis (along with other mercury compounds), although it is so toxic that sometimes the symptoms of its toxicity were confused with those of the ailment it was believed to treat (Pimple *et al.,* 2002). It is also used as a disinfectant. Blue mass, a pill or syrup in which mercury is the main ingredient, was prescribed throughout the 19th century for numerous conditions including constipation, depression, child-bearing and toothaches (Mayell, 2007). In the early 20th century, mercury was administered to children yearly as a laxative and dewormer, and it was used in teething powders for infants. The mercury-containing organohalide merbromin (sometimes sold as Mercurochrome) is still widely used but has been banned in some countries such as the U.S. (Wikipedia, 2004).

The first recorded account of animal experimentation on the toxicity of mercury came from Rhazes (al-Razi) in the 9th century and in the 11th century Avicenna (Ibn Sina) had the foresight to recommend the use of mercury only as an external remedy, and quicksilver ointments were used by the Arabs in the treating of skin diseases (Norn *et al.,* 2008).

2.1.3 Occurrence

Mercury is an extremely rare element in Earth's crust, having an average crustal abundance by mass of only 0.08 parts per million (ppm) (Ehrlich and Newman, 2008). Because it does not blend geochemically with those elements that constitute the majority of the crustal mass, mercury ores can be extraordinarily concentrated considering the element's abundance in ordinary rock. The richest mercury ores contain up to 2.5% mercury by mass, and even the leanest concentrated deposits are at least 0.1% mercury 12,000 times average crustal abundance. It is found either as a native metal (rare) or in cinnabar, corderoite, livingstonit and other minerals, with cinnabar (HgS) being the most common ore. Mercury ores usually occur in very young orogenic belts where rocks of high density are forced to the crust of Earth, often in hot springs or other volcanic regions. (Boulland, 2006; Burkholder and Johnson, 2008).

2.1.4 Uses of Mercury

Mercury is used in thermometers, barometers, manometers, sphygmomanometers, float valves, mercury switches, mercury relays, fluorescent lamps and other devices. Though, concerns about the element's toxicity have led to mercury thermometers and sphygmomanometers being largely phased out in clinical environments in favour of alternatives such as alcohol- or galinstan-filled glass thermometers and thermistor- or infrared-based electronic instruments. Likewise, mechanical pressure gauges and electronic strain gauge sensors have replaced mercury sphygmomanometers. (Hammond, 2005).

Mercury remains in use in scientific research applications and in amalgam material for dental restoration in some locales. It is used in lighting: electricity passed through mercury

vapor in a fluorescent lamp produces short-wave ultraviolet light which then causes the phosphor in the tube to fluoresce, making visible light (Hammond, 2005).

2.1.5 Mercury Poisoning

Mercury poisoning (also known as hydrargyria or mercurialism) is a type of metal poisoning and a medical condition caused by exposure to mercury or its compounds. All forms of mercury, except elemental liquid mercury (for which intravenous injection of a certain volume) produces toxicity or death with less than a gram. Elemental mercury does cause damage by blocking blood vessels. Mercury's zero oxidation state Hg^0 exists as vapor or as liquid metal, its mercurous state Hg^+ exists as inorganic salts, and its mercuric state Hg^{2+} may form either inorganic salts or organomercury compounds; the three groups vary in effects. Toxic effects include damage to the brain, kidneys and lungs (Clifton, 2007). Mercury poisoning can result in several diseases, including acrodynia (pink disease) (Bjorklund, 1995); Hunter-Russell syndrome (Tokuomi *et al.*, 1997) and Minamata disease (Davidson *et al.*, 2004).

Symptoms typically include sensory impairment (vision, hearing and speech), disturbed sensation and a lack of coordination. The type and degree of symptoms exhibited depend upon the individual toxin, the dose, and the method and duration of exposure. Common symptoms of mercury poisoning include peripheral neuropathy, presenting as paresthesia or itching, burning, pain, or even a sensation that resembles small insects crawling on or under the skin (formication); skin discoloration (pink cheeks, fingertips and toes); swelling; and desquamation (shedding or peeling of skin) (Horowitz *et al.*, 2002).

Mercury irreversibly inhibits selenium-dependent enzymes and may also inactivate S-adenosyl-methionine, which is necessary for catecholamine catabolism by catechol-o-methyl transferase. Due to the body's inability to degrade catecholamines (e.g. epinephrine), a person suffering from mercury poisoning may experience profuse sweating, tachycardia; persistently faster-than-normal heart beat, increased salivation, and hypertension (high blood pressure) (Horowitz *et al.,* 2002).

Affected children may show red cheeks, nose and lips; loss of hair, teeth, and nails, transient rashes, hypotonia (muscle weakness), and increased sensitivity to light. Other symptoms may include kidney dysfunction (e.g. Fanconi syndrome) or neuropsychiatric symptoms such as emotional lability, memory impairment, and / or insomnia. Thus, the clinical presentation may resemble pheochromocytoma or Kawasaki disease (Horowitz *et al.,* 2002).

The toxicity of mercury sources can be expected to depend on its nature. One mechanism of mercury toxicity involves its irreversible inhibition of selenoenzymes, such as thioredoxin reductase (Carvalho et al., 2007). Although it has many functions, thioredoxin reductase restores vitamins C and E, as well as a number of other important antioxidant molecules, back into their reduced forms, enabling them to counteract oxidative damage (Linster *et al.,* 2007). Since the rate of oxygen consumption is particularly high in brain tissues, production of reactive oxygen species (ROS) is accentuated in these vital cells, making them particularly vulnerable to oxidative damage and especially dependent upon the antioxidant protection provided by selenoenzymes. High mercury exposures deplete the amount of cellular selenium available for the biosynthesis of thioredoxin reductase and

other selenoenzymes that prevent and reverse oxidative damage which, if severe and long lasting, results in brain cell dysfunctions that can ultimately cause death (Ralston *et al.,* 2010);

In addition to the brain, metallic mercury is also deposited in the liver and kidneys (Danscher *et al.*, 1990). Acute poisoning with mercuric salts typically $HgCl_2$ generally targets the gastrointestinal tract and the kidneys. Extensive precipitation of enterocyte proteins occurs, with abdominal pain, vomiting, and bloody diarrhea with potential necrosis of the gut mucosa. This may produce death either from peritonitis or from septic or hypovolemic shock. Surviving patients commonly develop renal tubular necrosis with anuria (Barnes *et al.*, 1980).

Mercury in its various forms is particularly harmful to fetuses as an environmental toxin in pregnancy, as well as to infants. Women who have been exposed to mercury in substantial excess of dietary selenium intakes during pregnancy are at risk of giving birth to children with serious birth defects. Mercury exposures in excess of dietary selenium intakes in young children can have severe neurological consequences, preventing nerve sheaths from forming properly (Ralston and Raymond, 2010).

2.1.6 Sources of Mercury Exposure

The principal sources of exposure to mercury in the general population are ingestion and inhalation of mercury compounds from dental amalgams, and ingestion of fish (fresh water and marine) and seafoods which contain mercury, primarily as methylmercury (WHO, 1991; Richardson, 1996). Adults and children may also be exposed to small amounts of

elemental mercury from broken thermometers, barometers, fluorescent lights, electric switches and inhalation of atmospheric mercury (CEHPA, 2001).

In the environment, mercury comes from natural and anthropogenic sources (WHO, 1991). Mercury can be released into the air through geothermal activity and the weathering of mercury ore-containing rock. Anthropogenic sources of mercury, primarily from the combustion of fossil fuels, and incineration of medical and municipal waste, contribute greater than 80% of the mercury emitted from point sources according to the Agency for Toxic Substances Disease Registry (ATSDR, 1999) and 25% of overall (natural and anthropogenic) mercury emissions to the atmosphere (U.S. EPA, 1997). Based on the most recent inventory by the California Air Resources Board (CARB, 2000), annual statewide industrial emissions of mercury in 1998 were 9,714 lbs, and ambient air levels were 1.6 ng/m^3.

Once in the environment, interconversion between the different forms of mercury can occur. Conversion of inorganic mercury to methylmercury occurs primarily in microorganisms especially in aquatic systems. In the methylated form, mercury bioaccumulates up the food chain and can reach very high concentrations in some fishes, many of which are consumed by humans. The majority of exposure to mercury is dietary. The 50th percentile of mercury ingestion for the U.S. population is 1.4 μg/day (NRC, 2000).

In all, there are over 3000 industrial uses for mercury and its various compounds. From pulp and paper to floor waxes and vaccine preservatives, use of this powerful environmental poison is widespread. Ironically, despite grave concern about its toxicity, the greatest source of human exposure to mercury is from mercury-amalgam fillings that are still used for the vast majority of tooth restorations. Each year in the United States, over 100 million mercury amalgams are placed into the mouths of Americans—without a scintilla of evidence of safety (WHO, 1991).

2.1.7 Diagnosis of mercury poisoning

Diagnosis of elemental or inorganic mercury poisoning involves determining the history of exposure, physical findings, and an elevated body burden of mercury. Although, whole-blood mercury concentrations are typically less than 6 μg/L, diets rich in fish can result in blood mercury concentrations higher than 200 μg/L; it is not that useful to measure these levels for suspected cases of elemental or inorganic poisoning because of mercury's short half-life in the blood. If the exposure is chronic, urine levels can be obtained; 24-hour collections are more reliable than spot collections. It is difficult or impossible to interpret urine samples of patients undergoing chelation therapy, as the therapy itself increases mercury levels in the samples. Diagnosis of organic mercury poisoning differs in that whole-blood or hair analysis is more reliable than urinary mercury levels (Ibrahim *et al.*, 2006).

2.1.8 Prevention

Mercury poisoning can be prevented or minimized by eliminating or reducing exposure to mercury and mercury compounds. To that end, many governments and private groups have

made efforts to regulate heavily the use of mercury, or to issue advisories about its use. For example, the export from the European Union of mercury and some mercury compounds has been prohibited since the 15th of March, 2010 (EPPR, 2008).

2.1.9 Treatment

Identifying and removing the source of the mercury is crucial. Decontamination requires removal of clothes, washing skin with soap and water, and flushing the eyes with saline solution as needed (ATSDR, 2014).

Chelation therapy for acute inorganic mercury poisoning can be done with Dimercaptosuccinic acid (DMSA), 2,3-dimercapto-1-propanesulfonic acid (DMPS), D-penicillamine (DPCN), or dimercaprol (BAL) (Clifton, 2007). Only DMSA is FDA-approved for use in children for treating mercury poisoning. However, several studies found no clear clinical benefit from DMSA treatment for poisoning due to mercury vapor (Risher and Amler, 2005). No chelator for methylmercury or ethylmercury is approved by the FDA; DMSA is the most frequently used for severe methylmercury poisoning, as it is given orally, has fewer side-effects, and has been found to be superior to BAL, DPCN, and DMPS (Clifton, 2007). α-Lipoic acid (ALA) has been shown to be protective against acute mercury poisoning in several mammalian species when it is given soon after exposure; correct dosage is required, as inappropriate dosages increase toxicity. Although it has been hypothesized that frequent low dosages of ALA may have potential as a mercury chelator, studies in rats have been contradictory (Rooney, 2007). Glutathione and *N*-acetylcysteine (NAC) are recommended by some physicians, but have been shown to increase mercury concentrations in the kidneys and the brain (Rooney, 2007). Experimental findings have

demonstrated an interaction between selenium and methylmercury, but epidemiological studies have found little evidence that selenium helps to protect against the adverse effects of methylmercury (Watanabe, 2002).

2.1.10 Prognosis

Some of the toxic effects of mercury are partially or wholly reversible; either through specific therapy or through natural elimination of the metal after exposure has been discontinued. Autopsy findings point to a half-life of inorganic mercury in human brains of 27.4 years (Rooney, 2014). Heavy or prolonged exposure can do irreversible damage, in particular in fetuses, infants, and young children. Young's syndrome is believed to be a long-term consequence of early childhood mercury poisoning (Hendry *et al.*, 1993). Mercuric chloride may cause cancer as it has caused increases in several types of tumors in rats and mice, while methyl mercury has caused kidney tumors in male rats. The EPA has classified mercuric chloride and methyl mercury as possible human carcinogens (ATSDR, 1999; EPA, 2010).

2.2 Plants

2.2.1 Medicinal Plants

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Lichteman, 2004; Lai and Roy,

2004). Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects (Lichteman, 2004; Lai and Roy, 2004).

The use of plants as medicines predates written human history. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth, 2001). Some of the pharmaceuticals currently available to physicians are derived from plants that have a long history of use as herbal remedies. Examples of such drugs include aspirin, digoxin, quinine, and opium (Swain, 1968).

The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more common in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available.

Many herbs have shown positive results in-vitro, animal model or small-scale clinical tests (Srinivasan, 2005).

In 2002, the United States National Center for Complementary and Integrative Health of the National Institutes of Health began funding clinical trials into the effectiveness of herbal medicine. In a 2010 survey of 1000 plants, 356 had clinical trials published evaluating their pharmacological activities and therapeutic applications while 12% of the plants, although available in the Western market, had no substantial studies of their properties (Cravotto *et al.,* 2010).

2.2.2 *Phoenix dactylifera* (Date Palm)

Phoenix dactylifera L. (*P. dactylifera*) a diploid with 2n = 36, is a member of the monocot family Arecaceae classified as a dioecious tall evergreen tree (Zohary and Hopf, 1993). *P. dactylifera* have been cultivated in the Middle East since at least 6000 BC (Copley *et al.,* 2001). It is considered as native to countries around the Arabian Gulf (Chandra *et al.,* 1992). The Palm family is a symbol of prosperity and love to Muslims and its legend dates back to Judeo-Christian mythology (Al-Qarawi *et al.,* 2004). *P. dactylifera* fruits have been an important component of the diet in most of the arid and semiarid regions of the world (Biglari *et al.,* 2008).

P. dactylifera is believed to have been introduced into Nigeria in the early 8th century by Arab traders from North Africa. *P. dactylifera* fruits are a highly valued delicacy among

many communities in Nigeria, especially during ceremonies and festivals (Sani *et al.*, 2010).

The various parts of this plant are widely used in folk medicine for the treatment of various ailments. In fact, Muslims believe that "*He who eats seven dates every morning will not be affected by poison or magic on the day he eats them*" (Miller *et al.*, 2003). Dates fruits are widely used in traditional medicine for the treatment of various disorders e.g. memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders (Nadkarni, 1976), and as a detersive and astringent in intestinal troubles. It is also used in the treatment for sore throat, colds, bronchial asthma, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication (Barh and Mazumdar, 2008). Dates are a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium (Abdel-Hafez *et al.*, 1980). Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 (thiamine), B_2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib and Marshall, 2003).

Studies indicate that the aqueous extracts of dates have potent antioxidant activity (Mansouri *et al.*, 2003); many researchers have also documented the antioxidant property of *Phoenix dactylifera* (Mohamed and Al-Okbi, 2004; Allaith and Abdul, 2005; Al-Qarawi *et al.*, 2008). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic, sinapic acids, flavonoids and procyanidins (Gu *et al.*, 2003).

2.2.3 Taxonomy

The taxonomy of *P. dactylifera* is shown in **Table 2.2**

Table 2.2: Taxonomy of *P. dactylifera*

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Liliopsida*
Order	*Arecal.es*
Family	*Arecaceae*
Genus	*Phoenix*
Species	*Phoenix dactylifera*
Binomial name	*Phoenix dactylifera Linn*

(Vyawahare *et al.*, 2009).

2.2.4 Botanical Description

P. dactylifera is dioeciously, medium-sized tree, 15–25m tall, often clumped with several trunks from a single root system, but often growing singly as well, that is, is a multi-stemmed palm from which the suckers are usually removed to create single stemmed specimens. Trimmed in this manner the date palm will grow to heights of 45.7m. The broad gray trunk is patterned with diamond-shaped leaf scars and is up to 16 inches in diameter. The large greenish or bluish gray pinnate leaves are typically 5.5-6.1m long by 0.6m wide. They are arranged in a thick canopy up to 40 feet wide.

Leaflets are 0.3-0.6m long and arranged in V-shape ranks that run the length of the leaf stem. Leaflets near the base are modified into sharp 7.6-10.2cm spines. The yellow orange to red fruit, called 'dates', are oblong and about 3.8cm in length. The *P. dactylifera* fruit is a berry consisting of a single seed surrounded by a fibrous, parchment-like endocarp, a fleshy mesocarp and the fruit skin (pericarp). The fruit is attached to the spikelet by a perianth (calyx or cap) (Barreveld, 1993), as shown in **Figure 2.1**.

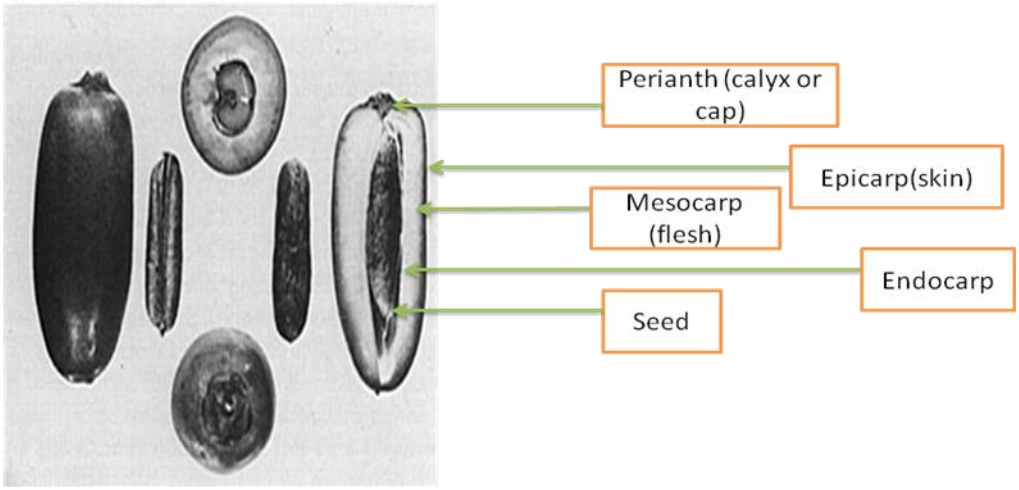


Figure 2.1 Date palm fruit and parts

(Morton *et. al*., 1987; Barreveld, 1993).

2.2.5 Varieties of Date Palm

Dates are oval-cylindrical, 3–7cm long, and 2–3cm diameter, and when unripe, range from bright red to bright yellow in colour, depending on variety as shown in **Table 2.3**.

Table 2.3: Description of some varieties of Date palm

S/No.	Variety	Brief description
1.	**'Barhi'**	Nearly cylindrical, light amber to dark brown when ripe; soft, with thick flesh and rich flavor; of superb quality.
2.	'Deglet Noor'	It is semi-dry, not very sweet; keeps well; much used for cooking. *The palm is high yielding but not very tolerant of rain and atmospheric humidity.*
3.	**'Halawy'** ('Halawi')	Soft, extremely sweet, small to medium; may shrivel during ripening unless the palm is well-watered. *It is especially tolerant of humidity.*
4.	**'Hayany'** ('Hayani')	The fruit is dark-red to nearly black; soft. *The palm is one of the most cold-tolerant; is sold fresh; is not easy to cure.*
5.	**'Khadrawy'** ('Khadrawi')	it is a soft date of the highest quality. It is early-ripening; does not keep too well. *This cultivar is the smallest edible date palm grown in the United States and it is fairly tolerant of rain and humidity; dark in color.*
6.	**'Khastawi'** ('Khustawi'; 'Kustawy')	Small in size; prized for dessert; keeps well. The palm is large and vigorous and produces its offshoots high on the trunk in California. *The fruit is resistant to humidity.*
7.	**'Medjool'**	Large, soft, and luscious.
8.	**'Saidy'** ('Saidi')	Soft, very sweet; palm is a heavy bearer; *needs a very hot climate.*

(Morton *et. al.*, 1987).

2.2.6 Nutritional Composition

Dates are of important nutritional value as shown in**Table 2.4**.

Table 2.4: Dried Dates (edible parts) Nutritional Value per 100 g

Energy	1,180 kJ (280 kcal)
Carbohydrates	75 g
Sugars	63 g
Dietary fibre	8 g
Fat	0.4 g
Protein	2.5 g
Water	21 g
Vitamin C	0.4 mg (1%)
Manganese	0.262 mg
Percentages are relative to US recommendations for adults. *Source*: USDA Nutrient database	

. (Sawaya *et al.*, 1983).

2.2.7 Phytochemistry

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent researches demonstrate that they can also protect humans against diseases.

Phytochemically, the whole plant (*P. dactylifera*) contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. The phenolic profile of the plant revealed the presence of mainly cinnamic acids (ferulic, sinapic and coumaric acids and their derivatives, such as 5-o-caffeoylshikimic acid also called as dactyliferic acid), flavonoid glycosides (luteolin, methyl luteolin, quercetin, and methyl quercetin), flavanols (catechin, epicatechin) (Seelig, 1974; Dowson, 1982; Biglari *et al.*, 2008).

Four free phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) and nine bound phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, and o-coumaric acid) were tentatively identified (Ziouti *et al.*, 1996; Eong *et al.*, 2006).

The thin layer chromatography (TLC) analysis revealed the presence of steroids namely cholesterol, stigmasterol, campesterol and α-sitosterol. Anthocyanins were detected only in fresh dates. The TLC analysis also showed that the major carotenoid pigment present in dates is lutein followed by α-carotene (Al-Farsi *et al.*, 2005; Boudries *et al.*, 2007).

Moreover the gas liquid chromatography of the date seed oil revealed the presence of oleic, lauric, palmitic, Capric, myristic, myristoleic, palmitoleic, stearic, linoleic and linolenic acids. Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib *et al.,* 1993). Enzymes such as phytase, invertase and peroxidase have been isolated in dates (Nadkarni, 1976). Presence of estrone has also been reported in the dried date seeds and pollens (Heftmann and Bennett, 1965; Bennett and Heftmann, 1966). The date palm pollen grains showed the presence of α-amirin, triterpenoids saponins and a crude gonadotropic substance (Mahran *et al.,* 1976). Other isolated chemical constituents include α-D glucan, heteroxylon, and galactomannans (Hussein *et al.,* 1998; Tabeta *et al.,* 2002; Ishurd *et al.,* 2003).

2.2.8 Medicinal Applications of *Phoenix dactylifera*

The water in which fresh dates are soaked for a while is a drink given to relieve alcohol intoxication. The milk in which clean and fresh dates are infused is a very nourishing and restorative drink to the children as well as adults especially during convalescence from fevers and smallpox. The sweet pulp of the date fruits is useful in dysentry. Date fruits are used as an ingredient in various aphrodiasiac and tonic confections. They are also useful in asthma. Paste made from ground seeds of date is said to be applied for opacity of cornea and to the head to relieve headache and hemicrania. The smoke produced from burning of date seeds in powder form is a useful fumigatory for piles (Anonymus, 1985).

Doctors advise that date fruits should be consumed as they promote expectoration, soothe the chest and prevent constipation (Anonymus, 1985). Dried fruit pounded and mixed with almonds, quince seeds, pistachia nuts, spices and sugar forms a nutritious formulation. Seeds roasted and ground into powder make a beverage like coffee called "date coffee". A fine paste of the seed of date fruit and the roots of *Achyranthus aspera*, applied to betel leaves like lime and made into small packets with clove, cardamom, catechu and betelnut powder is a popular antiperiodic remedy among Vaidyas for the prevention of augue which is preceeded by severe shivering. A gum or juice obtained from the stem and named "laghi" (Khajurnidaru) is used as a demulscent, diuretic and referigerant in genitourinary affections (Nadkarmi, 1976).

The liquid distillate obtained from spathe of *P. dactylifera* locally known as 'Maa Al-liqah' or 'Maa Al-Tiltal', water of the spathe is believed to have certain medical uses as it relieves abdominal gases and pain especially after heavy meal and claimed to have anti-spasmodic activity (Nadkarni, 1976). The flower of the plant is used as a purgative (Zoghet *et al.*, 2000). The pollen grains of date palm have been used in Egyptian local practice to improve fertility in women (Amin *et al.*, 1969).

Owning to the above mentioned medicinal applications of date palm, several researches have been conducted to scientifically validate these folk medicinal claims. Recently, many pharmacological studies (*in vitro and in vivo*) conducted on *P. dactylifera* have been demonstrated to have the following activities:

- **Anti-ulcer Activity**

Al-Qarawi *et al.,* (2005) carried out a study on the ameliorative effect of dates (*Phoenix dactylifera L*) on ethanol-induced gastric ulcer in rats. The work aimed at testing, in a rat model of ethanol-induced gastric ulceration, a local folk medicinal claim that dates are beneficial in gastric ulcers in humans. Aqueous and ethanolic extracts from date fruit and pits were given orally to rats at a dose of 4 ml/kg for 14 consecutive days. Gastric ulcer was induced by the administration of ethanol, 80% (1 ml/rat) by gastric intubation.

The results indicated that the aqueous and ethanolic extracts of the date fruit and, to a lesser extent, date pits, were effective in ameliorating the severity of gastric ulceration and mitigating the ethanol-induced increase in histamine and gastrin concentrations, and the decrease in mucin gastric levels. The ethanolic undialyzed extract was more effective than the rest of the other extracts used. It is postulated that the basis of the gastroprotective action of date extracts may be multi-factorial, and may include an antioxidant action.

- **Antioxidant Activity**

Phytochemicals from fruits have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative diseases in human (Javanmardi *et al.,* 2003). Various *in vitro* and *in vivo* antioxidant activity studies have been carried out on various extracts of different parts of *Phoenix dactylifera*. Studies conducted on antioxidant activity and phenolic content of various fruits of *Phoenix dactylifera* cultivated in Iran, Algeria and Bahrain demonstrated a linear relationship between antioxidant activity and the total phenolic content (TPC) of date fruit extract (Allaith *et al.,* 2005). Aqueous date extract was found to inhibit significantly the

lipid peroxidation and protein oxidation and also exhibited a potent superoxide and hydroxyl radical scavenging activity in a dose-dependent manner in an in vitro study (Dammak *et al.*, 2008).

Methanolic extract of *Phoenix dactylifera* seeds showed a significant increase in plasma levels of vitamin C, E and A, β-carotene and significant decrease in the elevated MDA levels due to the lipid peroxidation in adjuvant arthritis in rats (Mohamed and Al-Okbi, 2004). These findings suggests its possible use in diseases such as scurvy, ataxia and night blindness caused due to the deficiency of vitamins C, E and A respectively. Date seed oil was found to limit oxidative injuries induced by hydrogen peroxide in human skin organ culture which confirmed the potent free radical scavenging activity of the plant (Dammak *et al.*, 2008).

- **Anti-inflammatory Activity**

Oral administration of the methanolic and aqueous extracts of edible portion of *Phoenix dactylifera* fruits suppressed the swelling in the foot significantly by 67.8 and 61.3% respectively, while the methanolic extract of date seeds showed significant reduction by 35.5% in adjuvant arthritis in rats by mechanistically reducing ESR and plasma fibrinogen and normalizing the plasma level of antioxidants. Administration of the extracts also produced significant increase in body weight gain and food efficiency ratio (Mohamed and Al-Okbi, 2004).

- **Hematoprotective**

Abdul-Wahab *et al.* (2010) carried out a work to investigate the Preventive Effects of Ethanolic Extract of *Phoenix dactylifera l.* on Lead Induced Hematotoxicity in Rats. The

results showed that lead caused a significant decrease in hematocrit, RBC, WBC, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and lymphocyte and monocyte count; and significant increase in neutrophil count. These were, however, prevented in the extract of *Phoenix dactylifera* (EPD) treated groups. It is therefore concluded that oral admistration of EPD promotes blood's health and annuls lead induced hematotoxicity.

- **Hepatoprotective Activity**

Pre- and post-treatment with aqueous extract of date flesh or pits significantly reduced CCl_4 induced elevation in plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) enzymes and bilirubin concentration and ameliorated morphological and histological liver damage in rats. This study suggests that CCl4-induced liver damage in rats can be reversed by treatment of extracts from date flesh or pits. Moreover it can also be used prophylactically as a dymnamic liver support (Al-Qarawi *et al.,* 2004).

In another study the date flesh or pit extracts not only normalised the elevated glutamylγplasma activities of AST, ALT, ALP, lactate dehydrogenase (LDH), GT, enzymes and plasma concentration of bilirubin but also γtransferase (exhibited an enormous increase in the reduced serum levels of testosterone, alpha fetoprotien (AFP) and glucose in the thioacetamide induced chirrotic rats. The extracts also showed significant reduction in oxidative stress evidenced by significant rise in the hepatic malonaldehyde (MDA) levels and decline in hepatic glutathione levels by normalising them. This data suggest that the

daily oral consumption of an aqueous extract of the flesh of dates, and as a part of the daily diet ad libitum, was prophylactic to thioacetamide poisoning (Mohamed *et al.,* 2008).

- **Neuroprotective Activity**

Phoenix dactylifera provides a physical benefit that is crucial to the brain, called the neuroprotective or cerebroprotective effect. In general, this neuroprotective effect protects the brain from the destructive activity of the reactive oxygen species (ROS) that could come from the cell metabolism itself or from exogenous sources (Steinbrenner and Sies, 2009).

Date palm is an excellent antioxidant agent and this is due to the high concentrations of phenolic compounds, flavonoids and anthocyanins as well as the presence of selenoproteins (Baliga *et al.,* 2011). Antioxidant effects have been implicated for neuroprotective activity of *P. dactylifera* (Asadi-Shekaari *et al.,* 2008; Zangiabadi *et al.,* 2011; Kalantaripour *et al.,* 2012).

- **Nephroprotective Activity**

Al-Qarawi *et al.* (2008) studied the effect of the extracts of the flesh and pits of *Phoenix dactylifera* on gentamicin-induced nephrotoxicity in rats was investigated in which the extracts significantly reduced the increase in plasma creatinine and urea concentrations induced by gentamycin nephrotoxicity and ameliorated the proximal tubular damage. Antioxidant components in the date (e.g., melatonin, vitamin E, and ascorbic acid) were suggested to be the basis of the nephroprotection.

2.3 The Liver

The liver is a vital organ of vertebrates and some other animals (Abdel-Mesih *et al.,* 2010). In the human it is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion (CCS, 2015).

The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification (CCS, 2015). It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The gallbladder, a small pouch that sits just under the liver, stores bile produced by the liver (Tortora and Derrickson, 2008). The liver is a highly specialized tissue consisting of mostly hepatocytes which regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *et al*., 1993). Estimates regarding the organ's total number of functions vary, but textbooks generally cite it being around 500 (Zakim *et al.,* 2002).

There is currently no way to compensate for the absence of liver function in the long term, although liver dialysis techniques can be used in the short term. Liver transplantation is the only option for complete liver failure (*eMedicine,* 2015).

2.3.1 Embryology

Organogenesis, the development of the organs takes place from the third to the eighth week in human embryogenesis. The origins of the liver lie in both the ventral portion of the foregut endoderm (endoderm being one of the 3 embryonic germ layers) and the constituents of the adjacent septum transversum mesenchyme. In the human embryo, the hepatic diverticulum is the tube of endoderm that extends out from the foregut into the surrounding mesenchyme. The mesenchyme of septum transversum induces this endoderm to proliferate, to branch, and to form the glandular epithelium of the liver. A portion of the hepatic diverticulum (that region closest to the digestive tube) continues to function as the drainage duct of the liver, and a branch from this duct produces the gallbladder (Gilbert, 2000). Besides signals from the septum transversum mesenchyme, fibroblast growth factor from the developing heart also contributes to hepatic competence, along with retinoic acid emanating from the lateral plate mesoderm. The hepatic endodermal cells undergo a morphological transition from columnar to pseudostratified cells resulting in thickening into the early liver bud. Their expansion forms a population of the bipotential hepatoblasts (Berg *et al.*, 2010). Hepatic stellate cells are derived from mesenchyme (Clemente, 2011).

After migration of hepatoblasts into the septum transversum mesenchyme, the hepatic architecture begins to be established, with liver sinusoids and bile canaliculi appearing. The liver bud separates into the lobes. The left umbilical vein becomes the ductus venosus and the right vitelline vein becomes the portal vein. The expanding liver bud is colonized by hematopoietic cells. The bipotential hepatoblasts begin differentiating into biliary epithelial cells and hepatocytes. The biliary epithelial cells differentiate from hepatoblasts around portal veins, first producing a monolayer, and then a bilayer of cuboidal cells. In ductal

plate, focal dilations emerge at points in the bilayer, become surrounded by portal mesenchyme, and undergo tubulogenesis into intrahepatic bile ducts. Hepatoblasts not adjacent to portal veins instead differentiate into hepatocytes and arrange into cords lined by sinudoidal epithelial cells and bile canaliculi. Once hepatoblasts are specified into hepatocytes and undergo further expansion, they begin acquiring the functions of a mature hepatocyte, and eventually mature hepatocytes appear as highly polarized epithelial cells with abundant glycogen accumulation. In the adult liver, hepatocytes are not equivalent, with position along the portocentrovenular axis within a liver lobule dictating expression of metabolic genes involved in drug metabolism, carbohydrate metabolism, ammonia detoxification, and bile production and secretion. *β*-catenin has now been identified to be playing a key role in this phenomenon (Berg *et al.,* 2010).

2.3.2 Fetal Blood Circulation

In the growing fetus, a major source of blood to the liver is the umbilical vein which supplies nutrients to the growing fetus. The umbilical vein enters the abdomen at the umbilicus, and passes upward along the free margin of the falciform ligament of the liver to the inferior surface of the liver. There, it joins with the left branch of the portal vein. The ductus venosus carries blood from the left portal vein to the left hepatic vein and then to the inferior vena cava, allowing placental blood to bypass the liver (Moore and Dalley, 2006).

In the fetus, the liver develops throughout normal gestation, and does not perform the normal filtration of the infant liver. The liver does not perform digestive processes because the fetus does not consume meals directly, but receives nourishment from the mother via the placenta. The fetal liver releases some blood stem cells that migrate to the fetal thymus, so

initially the lymphocytes, called T-cells, are created from fetal liver stem cells. Once the fetus is delivered, the formation of blood stem cells in infants shifts to the red bone marrow.

After birth, the umbilical vein and ductus venosus are completely obliterated in two to five days; the former becomes the ligamentum teres and the latter becomes the ligamentum venosum. In the disease state of cirrhosis and portal hypertension, the umbilical vein can open up again (Moore and Dalley, 2006).

2.3.3 Gross Anatomy

Surfaces, Peritoneal reflections and Surfaces of the Liver

The liver has a convex diaphragmatic surface (anterior, superior, and some posterior) and a relatively flat or even concave visceral surface (posteroinferior), which are separated anteriorly by its sharp inferior border. The diaphragmatic surface of the liver is smooth and dome shaped, where it is related to the concavity of the inferior surface of the diaphragm. Subphrenic recesses superior extensions of the peritoneal cavity (greater sac) exist between diaphragm and the anterior and superior aspects of the diaphragmatic surface of the liver (Moore and Dalley, 2006).

The subphrenic recesses are separated into right and left recesses by the falciform ligament, which extends between the liver and the anterior abdominal wall. The portion of the supracolic compartment of the peritoneal cavity immediately inferior to the liver is the subhepatic space. The hepatorenal recess (hepatorenal pouch; Morison pouch) is the posterosuperior extension of the subhepatic space, lying between the right part of the visceral surface of the liver and the right kidney and suprarenal gland. The hepatorenal recess is a gravity-dependent part of the peritoneal cavity in the supine position; fluid

draining from the omental bursa flows into this recess. The hepatorenal recess communicates anteriorly with the right subphrenic recess. Recall that normally; all recesses of the peritoneal cavity are potential spaces only, containing only enough peritoneal fluid to lubricate the adjacent peritoneal membranes (Moore and Dalley, 2006).

The diaphragmatic surface of the liver is covered with visceral peritoneum, except posteriorly in the bare area of the liver, where it lies in direct contact with the diaphragm. The bare area is demarcated by the reflection of peritoneum from the diaphragm to it as the anterior (upper) and posterior (lower) layers of the coronary ligament. These layers meet on the right to form the right triangular ligament and diverge toward the left to enclose the triangular bare area. The anterior layer of the coronary ligament is continuous on the left with the right layer of the falciform ligament, and the posterior layer is continuous with the right layer of the lesser omentum. Near the apex (the left extremity) of the wedge-shaped liver, the anterior and posterior layers of the left part of the coronary ligament meet to form the left triangular ligament. The inferior vena cava (IVC) traverses a deep groove for the vena cava within the bare area of the liver (Moore and Dalley, 2006).

The visceral surface of the liver is covered with peritoneum, except at the fossa for the gallbladder and the porta hepatis, a transverse fissure where the vessels (portal vein, hepatic artery, and lymphatic vessels), the hepatic nerve plexus, and hepatic ducts that supply and drain the liver enter and leave it. In contrast to the smooth diaphragmatic surface, the visceral surface bears multiple fissures and impressions from contact with other organs. Two sagittal fissures, linked centrally by the transverse porta hepatis, form the letter H on the visceral surface. The right sagittal fissure is the continuous groove formed anteriorly by

the fossa for the gallbladder and posteriorly by the groove for the vena cava; the left sagittal fissure is the continuous groove formed anteriorly by the fissure for the ligamentum teres or round ligament, and posteriorly by the fissure for the ligamentum venosum. The round ligament of the liver is the fibrous remnant of the umbilical vein, which carried well-oxygenated and nutrient-rich blood from the placenta to the fetus; the round ligament and small paraumbilical veins course in the free edge of the falciform ligament. The ligamentum venosum is the fibrous remnant of the fetal ductus venosus, which shunted blood from the umbilical vein to the IVC, short-circuiting the liver (Moore and Persaud, 2003).

The lesser omentum, enclosing the portal triad (bile duct, hepatic artery, and portal vein) passes from the liver to the lesser curvature of the stomach and the first 2 cm of the superior part of the duodenum. The thick, free edge of the lesser omentum extends between the porta hepatis and the duodenum (the hepatoduodenal ligament) and encloses the structures that pass through the porta hepatis. The sheet-like remainder of the lesser omentum, the hepatogastric ligament, extends between the groove for the ligamentum venosum of the liver and the lesser curvature of the stomach (Moore and Persaud, 2003).

In addition to the fissures, impressions on (areas of) the visceral surface reflects the liver's relationship to the:

- Right side of the anterior aspect of the stomach (the gastric and pyloric areas).
- Superior part of the duodenum (the duodenal area).
- Lesser omentum (extends into the fissure for the ligamentum venosum).
- Gallbladder (fossa for gallbladder).

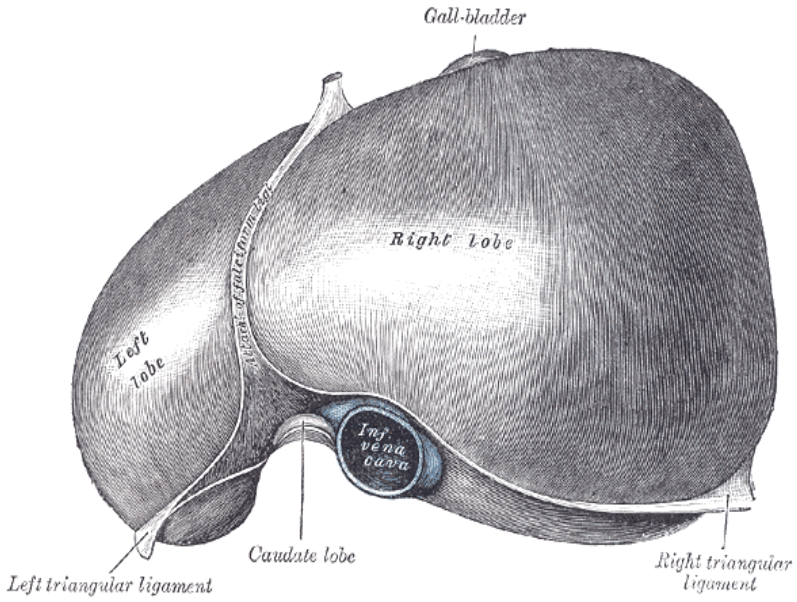
- Right colic flexure and right transverse colon (the colic area).
- Right kidney and suprarenal gland (the renal and suprarenal areas) (Moore and Dalley, 2006).

2.3.4 Anatomical Lobes of the Liver

Externally in humans, the liver is divided into two topographical (anatomical) lobes and two accessory lobes by the reflections of peritoneum from its surface, the fissures formed in relation to those reflections, and the vessels serving the liver and gallbladder. These superficial lobes are not true lobes as the term is generally used in relation to glands and are only secondarily related to the liver's internal architecture. The essentially midline plane defined by the attachment of the falciform ligament and the left sagittal fissure separates a large right lobe from a much smaller left lobe. On the slanted visceral surface, the right and left sagittal fissures surround and the transverse porta hepatis demarcates two accessory lobes (parts of the anatomic right lobe): the quadrate lobe anteriorly and inferiorly and the caudate lobe posteriorly and superiorly. The caudate lobe is so-named not because it is caudal in position (it is not) but because it often gives rise to a tail in the form of an elongated papillary process. A caudate process extends to the right, between the IVC and the portal hepatis, connecting the caudate and right lobes (Moore and Dalley, 2006), as shown in **Figure 2.2.**

2.3.5 Functional Subdivision of the Liver

Although not distinctly demarcated internally, where the parenchyma appears continuous, the liver has functionally independent right and left livers (parts or portal lobes) that are much more equal in size than the anatomical lobes; however, the right liver is still somewhat larger. Each part receives its own primary branch of the hepatic artery and portal vein and is drained by its own hepatic duct. The caudate lobe may in fact be considered a third liver; its vascularization is independent of the bifurcation of the portal triad (it receives vessels from both bundles) and is drained by one or two small hepatic veins, which enter directly into the IVC distal to the main hepatic veins. The liver can be further subdivided into four divisions and then into eight surgically resectable hepatic segments, each served independently by a secondary or tertiary branch of the portal triad (Moore and Dalley, 2006), as shown in **Figure 2.2.**



Grey, 1918.

Figure 2.2a

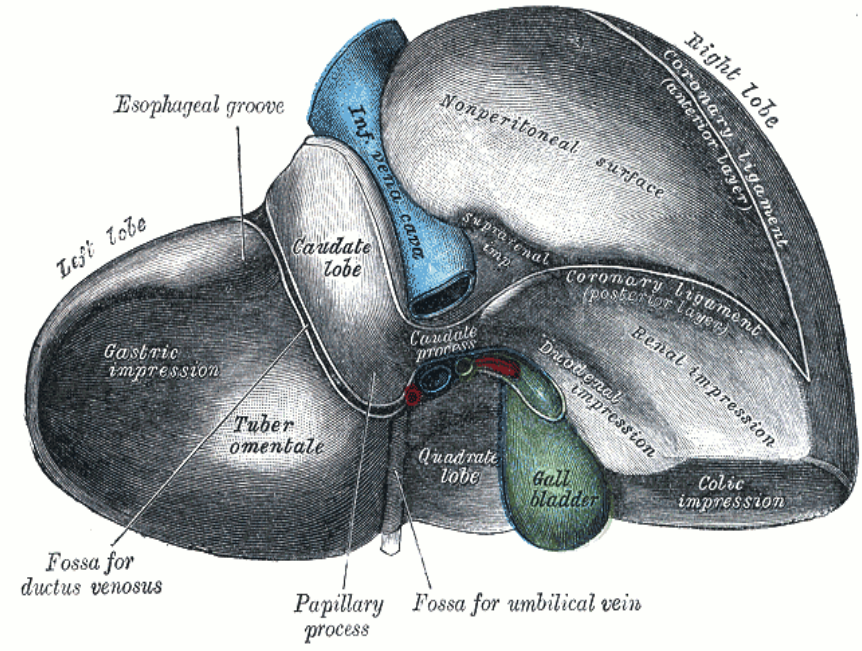


Figure 2.2b

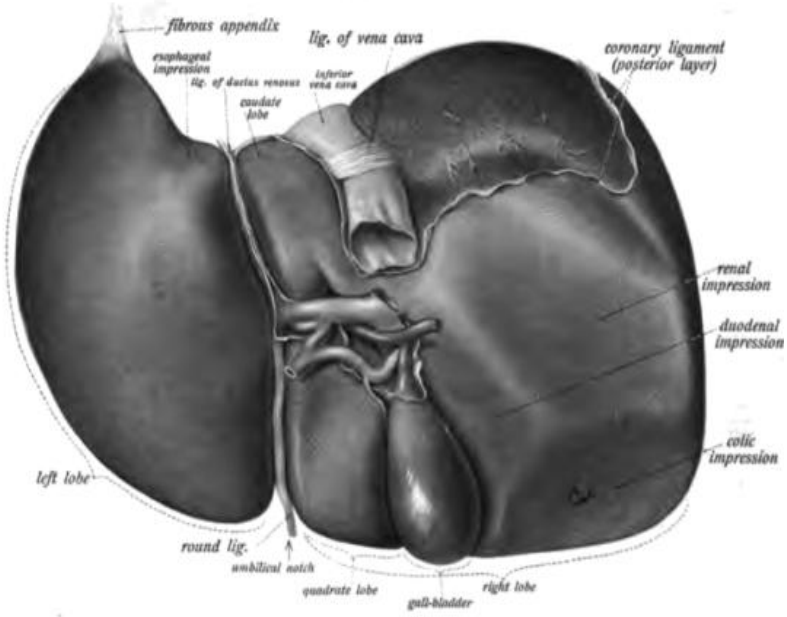


Figure 2.2 Gross anatomical features of the liver.

Grey, 1918.

Figure 2.2c

2.3.6 Blood Vessels of the Liver

The liver, like the lungs, has a dual blood supply (afferent vessels): a dominant venous source and a lesser arterial one. The portal vein brings 75-80% of the blood to the liver. Portal blood, containing about 40% more oxygen than blood returning to the heart from the systemic circuit, sustains the liver parenchyma (liver cells or hepatocytes). The portal vein carries virtually all of the nutrients absorbed by the alimentary tract (except lipids, which bypass the liver in the lymphatic system) to the sinusoids of the liver. Arterial blood from the hepatic artery, accounting for only 20-25% of blood received by the liver, is distributed initially to non-parenchymal structures, particularly the intrahepatic bile ducts (Moore and Dalley, 2006).

The portal vein, a short, wide vein, is formed by the superior mesenteric and splenic veins posterior to the neck of the pancreas and ascends anterior to the IVC as part of the portal triad in the hepatoduodenal ligament. The hepatic artery, a branch of the celiac trunk, may be divided into the common hepatic artery, from the celiac trunk to the origin of the gastroduodenal artery, and the hepatic artery proper, from the origin of the gastroduodenal artery to the bifurcation of the hepatic artery. At or close to the porta hepatis, the hepatic artery and portal vein terminate by dividing into right and left branches; these primary branches supply the right and left livers, respectively. Within each part, the simultaneous secondary branchings of the portal vein and hepatic artery (portal pedicles) are consistent enough to supply the medial and lateral divisions of the right and left liver, with three of the four secondary branches undergoing further (tertiary) branchings to supply independently seven of the eight hepatic segments. Between the divisions are the right, intermediate (middle), and left hepatic veins, which are intersegmental in their distribution and function,

draining parts of adjacent segments. The hepatic veins, formed by the union of collecting veins that in turn drain the central veins of the hepatic parenchyma, open into the IVC just inferior to the diaphragm. The attachment of these veins to the IVC helps hold the liver in position (Moore and Dalley, 2006).

2.3.7 Lymphatic Drainage of the Liver

The liver is a major lymph-producing organ. Between one quarter and one half of the lymph received by the thoracic duct comes from the liver. The lymphatic vessels of the liver occur as superficial lymphatics in the subperitoneal fibrous capsule of the liver (Glisson capsule), which forms its outer surface, and as deep lymphatics in the connective tissue, which accompany the ramifications of the portal triad and hepatic veins. Most of the lymph is formed in the perisinusoidal spaces (of Disse) and drains to the deep lymphatics in the surrounding intralobular portal triads. Superficial lymphatics from the anterior aspects of the diaphragmatic and visceral surfaces and the deep lymphatic vessels accompanying the portal triads converge toward the porta hepatis. They drain to the hepatic lymph nodes scattered along the hepatic vessels and ducts in the lesser omentum. Efferent lymphatic vessels from the hepatic nodes drain into celiac lymph nodes, which in turn drain into the chyle cistern, a dilated sac at the inferior end of the thoracic duct. Superficial lymphatics from the posterior aspects of the diaphragmatic and visceral surfaces of the liver drain toward the bare area of the liver. Here they drain into phrenic lymph nodes, or join deep lymphatics that have accompanied the hepatic veins converging on the IVC, and pass with this large vein through the diaphragm to drain into the posterior mediastinal lymph nodes. Efferent vessels from these nodes join the right lymphatic and thoracic ducts. A few lymphatic vessels follow different routes:

- From the posterior surface of the left lobe toward the esophageal hiatus of the diaphragm to end in the left gastric lymph nodes.
- From the anterior central diaphragmatic surface along the falciform ligament to the parasternal lymph nodes.
- Along the round ligament of the liver to the umbilicus and lymphatics of the anterior abdominal wall (Moore and Dalley, 2006).

2.3.8 Innervation of the Liver

The nerves of the liver are derived from the hepatic plexus, the largest derivative of the celiac plexus. The hepatic plexus accompanies the branches of the hepatic artery and portal vein to the liver. It consists of sympathetic fibers from the celiac plexus and parasympathetic fibers from the anterior and posterior vagal trunks. Nerve fibers accompany the vessels and biliary ducts of the portal triad. Other than vasoconstriction, their function is unclear (Moore and Dalley, 2006).

2.3.9 Histology of the Liver

Microscopically, each liver lobe is seen to be made up of hepatic lobules. The lobules are roughly hexagonal, and consist of plates of hepatocytes radiating from a central vein (Elaine and Katja, 2012). The central vein joins to the hepatic vein to carry blood out from the liver. A distinctive component of a lobule is the portal triad, which can be found running along each of the lobule's corners. The portal triad, misleadingly named, consists of five structures: a branch of the hepatic artery, a branch of the hepatic portal vein, and a bile duct,

as well as lymphatic vessels and a branch of the vagus nerve (Elaine and Katja, 2012). Between the hepatocyte plates are liver sinusoids, which are enlarged capillaries through which blood from the hepatic portal vein and hepatic artery enters via the portal triads, then drains to the central vein (Kmiec, 2001).

Histology, the study of microscopic anatomy, shows two major types of liver cell: parenchymal cells and non-parenchymal cells. 70–85% of the liver volume is occupied by parenchymal hepatocytes. Non-parenchymal cells constitute 40% of the total number of liver cells but only 6.5% of its volume (Kmiec, 2001). The liver sinusoids are lined with two types of cell, sinusoidal endothelial cells, and phagocytic Kupffer cells (Pocock, 2006). Hepatic stellate cells are non-parenchymal cells found in the space of Disse, between a sinusoid and a hepatocyte (Kmiec, 2001). Additionally, intrahepatic lymphocytes are often present in the sinusoidal lumen (Kmiec, 2001).

2.3.10 Functions of the Liver

The major functions of the liver may be summarised as follows:

- **Fat metabolism**
    - Oxidising triglycerides to produce energy.
    - Synthesis of plasma lipoproteins.
    - Synthesis of cholesterol and phospholipids.

- **Carbohydrate metabolism**
    - Converting carbohydrates and proteins into fatty acids and triglycerides.

- Regulation of blood glucose concentration by glycogenesis, glycogenolysis, glyconeogenesis

- **Protein metabolism**
  - Synthesis of plasma proteins, including albumin and clotting factors.
  - Synthesis of non-essential amino acids.
  - Detoxication of metabolic waste products, e.g. deamination of amino acids and production of urea.

- **Storage**
  - Storage of glycogen, vitamins and iron.

- **Intermediary metabolism**
  - Detoxification of various drugs and toxins, such as alcohol.

- **Secretion**
  - Synthesis and secretion of bile; bile contains many of the products of the above processes (Young *et al.,* 2007).

- **Endocytosis**
  - Phagocytosis of old red blood cells and certain bacteria (Moore and Persuade, 2011).

2.3.11 Liver Enzymes and Liver Function Test

There are several enzymes in the body. There are four (4) separate liver enzymes which are included in most of the routine laboratory tests. They are aspartate aminotransferase (AST) and alanine transaminase (ALT) and alkaline phosphate (ALP) and gamma-glutamyltransferase (GGT), which are known together as chlorostatic liver enzymes. Elevations of these enzymes can indicate the presence of liver disease. AST and ALT are jointly known as transaminases. They are associated with inflammation and/or injury to liver cells, a condition known as hepatocellular liver injury. Damage of liver typically results in a leak of AST and ALT into the blood stream. Because AST is found in many other organs beside the liver, such as the kidneys, muscles and the heart; having a high level of AST does not always but often indicate a liver problem. Sometimes, even vigorous exercises may elevate the AST level in the body. On the other hand, since ALT is primarily found in the liver, high levels of ALT always indicates that there is a problem with the liver, however, a normal ALT level does not necessarily mean that the organ is normal. These enzymes are the major liver biomarkers (Palmer, 2002).

The AST/ALT ratio shows the concentrations of AST and ALT in the blood of a human or animal. It is measured with a blood test and is sometimes useful in medical diagnosis to differentiate between causes of liver damage, or hepatotoxicity (Nyblom *et al.*, 2004).

Most causes of liver cell injury are associated with an AST that is lower than the ALT. However, an AST to ALT ratio of 2:1 or greater is suggestive of alcoholic liver disease, particularly in the setting of an elevated gamma-glutamyl transferase (Moussavian *et al.*, 1985).

2.4 The Kidney

The kidneys are bean-shaped organs that serve several essential regulatory roles in vertebrates. They remove excess organic molecules from the blood, and it is by this action that their best-known function is performed: the removal of waste products of metabolism. Kidneys are essential to the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure via maintaining the salt and water balance. They serve the body as a natural filter of the blood, and remove water-soluble wastes which are diverted to the bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium. They are also responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol and erythropoietin. An important enzyme renin is also produced in the kidneys which act in negative feedback (Wikipedia Encyclopedia, WE, 2016).

Located at the rear of the abdominal cavity in the retroperitoneal space, the kidneys receive blood from the paired renal arteries, and drain into the paired renal veins. Each kidney excretes urine into a ureter which empties into the bladder (WE, 2016).

Renal physiology is the study of kidney function, while nephrology is the medical specialty concerned with kidney diseases. Diseases of the kidney are diverse, but individuals with kidney disease frequently display characteristic clinical features. Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes, renal cysts, acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction (Cotran *et al*., 2005). Various cancers of the kidney exist. The most common adult renal cancer is renal cell carcinoma. Cancers, cysts, and some other renal

conditions can be managed with removal of the kidney. This is known as nephrectomy. When renal function, measured by the glomerular filtration rate, is persistently poor, dialysis and kidney transplantation may be treatment options. Although they are not normally harmful, kidney stones can be extremely painful (WE, 2016).

2.4.1 Embryology

Kidney development, or nephrogenesis, describes the embryologic origins of the kidney, a major organ in the urinary system. It is often considered in the broader context of the development of the urinary and reproductive organs (Bruce, 2004).

The development of the kidney proceeds through a series of successive phases, each marked by the development of a more advanced kidney: the pronephros, mesonephros, and metanephros. The pronephros is the most immature form of kidney, while the metanephros is most developed. The metanephros persists as the definitive adult kidney (Bruce, 2004).

- **Pronephros**

The pronephros develops in the cervical region of the embryo. During approximately day 22 of human gestation, the paired pronephri appear towards the cranial end of the intermediate mesoderm. In this region, epithelial cells arrange themselves in a series of tubules called nephrotomes and join laterally with the pronephric duct. This duct is fully contained within the embryo and thus cannot excrete filtered material outside the embryo; therefore the pronephros is considered nonfunctional in mammals (Bruce, 2004).

- **Mesonephros**

The development of the pronephric duct proceeds in a cranial-to-caudal direction. As it elongates caudally, the pronephric duct induces nearby intermediate mesoderm in the

thoracolumbar area to become epithelial tubules called mesonephric tubules. Each mesonephric tubule receives a blood supply from a branch of the aorta, ending in a capillary tuft analogous to the glomerulus of the definitive nephron. The mesonephric tubule forms a capsule around the capillary tuft, allowing for filtration of blood. This filtrate flows through the mesonephric tubule and is drained into the continuation of the pronephric duct, now called the mesonephric duct or Wolffian duct. The nephrotomes of the pronephros degenerate while the mesonephric duct extends towards the most caudal end of the embryo, ultimately attaching to the cloaca. The mammalian mesonephros is similar to the kidneys of aquatic amphibians and fishes (Bruce, 2004).

- **Metanephros**

During the fifth week of gestation, the mesonephric duct develops an outpouching, the ureteric bud, near its attachment to the cloaca. This bud, also called the metanephrogenic diverticulum, grows posteriorly and towards the head of the embryo. The elongated stalk of the ureteric bud, called the metanephric duct, later forms the ureter. As the cranial end of the bud extends into the intermediate mesoderm, it undergoes a series of branchings to form the collecting duct system of the kidney. It also forms the major and minor calyces and the renal pelvis.

The portion of undifferentiated intermediate mesoderm in contact with the tips of the branching ureteric bud is known as the metanephrogenic blastema. Signals released from the ureteric bud induce the differentiation of the metanephrogenic blastema into the renal tubules. As the renal tubules grow, they come into contact and join with connecting tubules of the collecting duct system, forming a continuous passage for flow from the renal tubule to the collecting duct. Simultaneously, precursors of vascular endothelial cells begin to take

their position at the tips of the renal tubules. These cells differentiate into the cells of the definitive glomerulus.

In humans, all of the branches of the ureteric bud and the nephronic units have been formed by 32 to 36 weeks of gestation. However, these structures are not yet mature, and will continue to mature after birth. Once matured, humans have an estimated two million nephrons (approximately 1,000,000 per kidney) but this number is highly variable ranging widely from approximately 300,000 to over 2 million per kidney (Bruce, 2004).

- **Migration**

After inducing the metanephric mesenchyme the lower portions of the nephric duct will migrate caudally (downward) and connect with the bladder, thereby forming the ureters. The ureters will carry urine from the kidneys to the bladder for excretion from the fetus into the amniotic sac. As the fetus develops, the torso elongates and the kidneys rotate and migrate upwards within the abdomen which causes the length of the ureters to increase (Bruce, 2004).

2.4.2 Gross Anatomy

In humans, the ovoid kidneys remove excess water, salts, and wastes of protein metabolism from the blood while returning nutrients and chemicals to the blood. They lie retroperitoneally on the posterior abdominal wall, one on each side of the vertebral column at the level of the T12-L3 vertebrae. The right kidney usually lies slightly inferior to the left kidney, probably owing to its relationship to the liver. During life, the kidneys are reddish brown and measure approximately 10 cm in length, 5 cm in width, and 2.5 cm in thickness. Superiorly, the kidneys are associated with the diaphragm, which separates them from the

pleural cavities and the 12th pair of ribs. More inferiorly, the posterior surfaces of the kidney are related to the quadratus lumborum muscle. The subcostal nerve and vessels and the iliohypogastric and ilioinguinal nerves descend diagonally across the posterior surfaces of the kidneys. The liver, duodenum, and ascending colon are anterior to the right kidney. The right kidney is separated from the liver by the hepatorenal recess. The left kidney is related to the stomach, spleen, pancreas, jejunum, and descending colon (Moore and Persaud, 2003).

At the concave medial margin of each kidney is a vertical cleft, the renal hilum, where the renal artery enters and the renal vein and renal pelvis leave the renal sinus. At the hilum, the renal vein is anterior to the renal artery, which is anterior to the renal pelvis. The renal hilum is the entrance to a space within the kidney, the renal sinus, which is occupied by the renal pelvis, calices, vessels, and nerves and a variable amount of fat. Each kidney has anterior and posterior surfaces, medial and lateral margins, and superior and inferior poles. However, because of the protrusion of the lumbar vertebral column into the abdominal cavity, the kidneys are obliquely placed, lying at an angle to each other. Consequently, the transverse diameter of the kidneys is foreshortened in anteroposterior (AP) radiographs. The lateral margin of each kidney is convex, and the medial margin is concave where the renal sinus and renal pelvis are located. The indented medial margin gives the kidney a somewhat bean-shaped appearance (Moore and Persaud, 2003).

The renal pelvis is the flattened, funnel-shaped expansion of the superior end of the ureter. The apex of the renal pelvis is continuous with the ureter. The renal pelvis receives two or three major calices (calyces), each of which divides into two or three minor calices. Each

minor calyx is indented by the renal papilla, the apex of the renal pyramid, from which the urine is excreted. In living persons, the renal pelvis and its calices are usually collapsed (empty). The pyramids and their associated cortex form the lobes of the kidney. The lobes are visible on the external surfaces of the kidneys in fetuses, and evidence of the lobes may persist for some time after birth (Moore and Persaud, 2003), as shown in **Figure 2.3**.

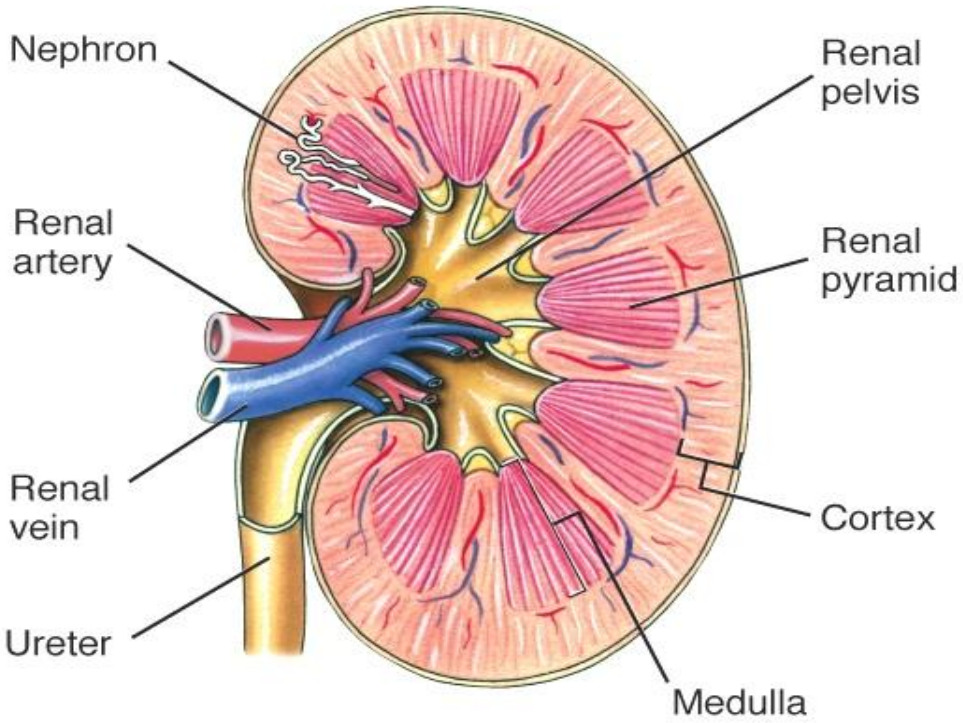


Figure 2.3 Gross anatomical features of the kidney

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2.4.3 Blood Supply, Venous Drainage amd Lymphatics of the Kidney

The renal arteries arise at the level of the IV disc between the L1 and the L2 vertebrae. The longer right renal artery passes posterior to the IVC. Typically, each artery divides close to the hilum into five segmental arteries that are end arteries (i.e., they do not anastomose significantly with other segmental arteries, so that the area supplied by each segmental artery is an independent, surgically resectable unit or renal segment). Segmental arteries are distributed to the renal segments as follows:

- The superior (apical) segment is supplied by the superior (apical) segmental artery; the anterosuperior and anteroinferior segments are supplied by the anterosuperior segmental and anteroinferior segmental arteries; and the inferior segment is supplied by the inferior segmental artery. These arteries originate from the anterior branch of the renal artery.
- The posterior segmental artery, which originates from a continuation of the posterior branch of the renal artery, supplies the posterior segment of the kidney (Moore and Dalley, 2006).

Several renal veins drain each kidney and unite in a variable fashion to form the right and left renal veins. The right and left renal veins lie anterior to the right and left renal arteries. The longer left renal vein receives the left suprarenal vein, the left gonadal (testicular or ovarian) vein, and a communication with the ascending lumbar vein, then passes anterior to the aorta. Each renal vein drains into the IVC (Moore and Dalley, 2006).

The renal lymphatic vessels follow the renal veins and drain into the right and left lumbar (caval and aortic) lymph nodes (Moore and Dalley, 2006).

2.4.4 Histology of the Kidney

Glomeruli and the tubular system are both part of the basic functional unit of the kidney, the nephron. The glomerulus is the round blind beginning of the nephron. It is invaginated by a tuft of capillaries at the vascular pole of the glomerulus. The anatomical glomerulus is enclosed by two layers of epithelium, Bowman's capsule. Cells of the outer or parietal layer of Bowman's capsule form a simple squamous epithelium. Cells of the inner layer, podocytes in the visceral layer, are extremely complex in shape. Small foot-like processes, pedicles, of their cytoplasm form a fenestrated epithelium around the fenestrated capillaries of the glomerulus (Slomianka, 2009).

The openings between the pedicles are called filtration slits. They are spanned by a thin membrane, the filtration slit membrane. Between the podocytes and the endothelial cells of the capillaries we find a comparatively thick basal lamina, which can be subdivided into an outer lamina (rara externa), a middle lamina (densa) and an inner lamina (rara interna). The basal lamina and the slit membranes form the glomerular filtration barrier, which prevents some large molecules from entering the capsular space between the outer and inner epithelial layers of Bowman's capsule. Mesangial cells in the glomerulus form the connective tissue that gives structural support to podocytes and vessels (Slomianka, 2009).

The tubular system can be divided into proximal and distal tubules, which in turn have convoluted and straight portions. Intermediate tubules connect the proximal and distal tubules. Running from the cortex of the kidney towards the medulla (descending), then turning and running back towards the cortex (ascending), the tubules form the loop of Henle (Slomianka, 2009).

The proximal tubule is the longest section of the nephron (about 14 mm). The convoluted part of the proximal tubules coils close to the glomerulus in the cortex. The diameter of proximal tubules is 65 µm. Their walls are formed by a low columnar epithelium. The eosinophilic cells of the epithelium have a wide brush border (long microvilli) and are active in endocytosis. They almost completely reabsorb substances of nutritional value from the glomerular filtrate mainly glucose, amino acids, protein and vitamins (Slomianka, 2009).

The straight portion of the proximal tubule merges with the intermediate tubule thin segment of the loop of Henle. A flattened, only 1-2 µm high epithelium forms the intermediate tubule, which is only 15 µm wide. Descending parts of the straight proximal and intermediate tubules are permeable to water but not to solutes (Slomianka, 2009).

The thin segment of Henle's loop leads into the straight part of the distal tubule, which is formed by low cuboidal cells without a brush border. A few short microvilli are present, but they are difficult to see in the light microscope. The diameter of the tubule expands to 35 µm. Epithelial cells in the ascending parts of the intermediate and straight distal tubules cells transport chloride (active) and sodium ions (passive) out of the tubular lumen into the surrounding peritubular space. The epithelium cannot be penetrated by water. Consequently, the transport of ions over the epithelium sets up a gradient in osmotic pressure, which serves as driving force in the further concentration of the urine.

The straight portion of the distal tubule contacts the glomerulus forming the macula densa. Thereafter, the distal tubule forms its convoluted portion (about 5 mm long). Cells in the distal tubulus are sensitive to the hormone aldosterone, which is produced in the zona glomerulosa of the adrenal glands. Aldosterone stimulates the active resorption of sodium ions and the excretion of potassium ions (Slomianka, 2009).

The convoluted distal tubule merges, via connecting tubules, with the collecting ducts. In the presence of

antidiuretic hormone (ADH), the epithelia of the collecting ducts are permeable to water but not to sodium ions. Osmotic forces move water out of the lumen of the tubules as they pass through the medulla, where cells of the ascending intermediate and straight distal tubules of the loop of Henle have established high concentrations of sodium in the extracellular space. Collecting ducts merge to form papillary ducts (of Bellini), which terminate on the tips of the renal papilla and empty into a distended, funnel shaped part (minor calyx) of the ureter (Slomianka, 2009).

As mentioned above, the distal tubule contacts the glomerulus forming a specialized section of tubular epithelium, the macula densa. At the point of contact with the glomerulus, the distal tubule is always in close contact with the efferent and afferent arterioles of the glomerulus (Slomianka, 2009).

Other parts of the juxtaglomerular apparatus are extraglomerular mesangial cells and the juxtaglomerular cells surrounding the afferent arteriole (modified smooth muscle cells), which produce and secrete renin. Renin activates angiotensinogen, a precursor found in the bloodstream, leading to the formation of angiotensin I, which is converted to angiotensin II. Angiotensin II is the most potent vasoconstrictor known. It also stimulates the secretion of aldosterone (Slomianka, 2009).

Different theories exist that try to explain the interactions between the cells that eventually lead to the release of renin. One of them, the baroreceptor theory, assumes that the juxtaglomerular cells function as stretch receptors (high blood pressure would inhibit the release of renin). Another theory, the macula densa theory, claims that the secretion of renin is regulated by the composition of the fluid in the distal tubule and/or the afferent arteriole (low sodium would increase in the release of renin) (Slomianka, 2009).

- **Excretory Passages**

The minor calyces merge to form major calyces within the kidney, which in turn merge to form the renal

pelvis (still within the kidney). The urine flows through these structures to the ureter and is channeled to the bladder (Slomianka, 2009).

The basic structure of all these components is the same. The mucosa is lined with a transitional epithelium, which occurs exclusively in the urinary system. The epithelium is virtually impenetrable to any components of the urine, which consequently does not change in composition as it passes through the excretory passages. The lamina propria consists mainly of dense connective tissue, with many bundles of coarse collagenous fibres. The muscularis usually consists of an inner longitudinal and outer circular layer of smooth muscle cells. In lower parts of the ureter and the bladder an additional outer longitudinal layer of muscles is added to the first two (Slomianka, 2009).

The bladder is finally emptied through the urethra. Initially, the urethra is lined by a transitional epithelium in males and females. In males, it is replaced by a pseudostratified or stratified columnar epithelium below the openings of the ejaculatory ducts into the urethra. The distal parts of the female urethra and the distal end of the male urethra are lined by a stratified squamous epithelium. The lamina propria contains loose connective tissue. Smooth muscle cells in the muscularis are mainly oriented longitudinally. They are surrounded, in the middle part of the urethra (below the prostate in males), by striated muscle cells of the sphincter urethra (Slomianka, 2009).

2.4.5 Functions of the Kidney

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, hormone secretion, osmolality regulation, reabsorbtion of vital nutrients, excretion of waste and blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions; these include renin, angiotensin II,

aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others (Slomianka, 2009).

Many of the kidney's functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron. Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that eventually becomes urine. The kidney generates 180 liters of filtrate a day, while reabsorbing a large percentage, allowing for the generation of only approximately 2 liters of urine. Reabsorption is the transport of molecules from this ultrafiltrate and into the blood. Secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine (Slomianka, 2009).

2.4.6 Kidney Function Test

Healthy kidneys remove wastes and excess fluid from the blood. Blood tests show whether the kidneys are failing to remove wastes. Kidney function tests can show how quickly body wastes are being removed and whether the kidneys are also leaking abnormal amounts of protein (National Kidney Foundation, NKD, 2016). Some of the parameters assessed in kidney function test are:

- **Serum Creatinine**

Creatinine is a waste product that comes from meat protein in the diet and also comes from the normal wear and tear on muscles of the body. Creatinine levels in the blood can vary, and each laboratory has its own normal range. In many labs the normal range is 0.6 to 1.2 mg/dl. Higher levels may be a sign that the kidneys are not working properly. As kidney disease progresses, the level of creatinine in the blood increases (NKD, 2016)

- **Blood Urea Nitrogen (BUN)**

Urea nitrogen also is produced from the breakdown of food protein. A normal BUN level is between 7 and 20 mg/dl. As kidney function decreases, the BUN level increases (NKD 2016).

2.5 Silymarin

Silymarin is a flavonolignan that has been introduced fairly recently as a hepatoprotective agent. It is the most well known compound of the flavanoids. It is extracted from the seed and fruit of *silibum marianum* (Compositae) and in reality is a mixture of three (3) structural components: silibinin (most active), silydianine and silychristine (Valenzuela and Garrido, 1994; Morazzoni and Bombardelli, 1995).

Silymarin, derived from the milk thistle plant named silybum marianum has been used since time past as a natural remedy for combating liver diseases. *Silybum marianum* belongs to the Aster family (Asteraceae or Compositeae), which includes daisies and thistle. Silymarin has been used for centuries to the liver, spleen, and gall bladder disorders (Shaker *et al.*, 2010). As a hepatoprotective agent, Silymarin is used for oral treatment of toxic liver damage and for the therapy of chronic inflammatory liver diseases (Flora *et al.*, 1998). It also possesses anti-cancer (Zi *et al.,* 1997), anti-inflammatory (De La Puerto, 1996) and anti-diabetic (Maghrani *et al.,* 2004) properties.

Silymarin is an antioxidant flavanoid with molecular formula $C_{25}H_{22}O_{10}$ (Merlini *et al.,* 1979) consisting of a mixture of three (3) isomers isolated from seeds of milk thistle which have properties for protecting the liver from or clearing it of toxins, and used in dietary supplements and herbal remedies. Other names of Silymarin are**:** Cardui mariae, Carduus marianum, Holy thistle, Lady's thistle, Legalon, Marian thistle,

Mariendistle, Mary thistle, Our lady's thistle, Silimarina, Silybin, Silybum marianum, St. Mary thistle.

2.5.1 Pharmacodynamics

The following are the major pharmacodynamic activities of Silymarin: stimulation of liver regeneration, inhibition of cytochrome P450, activity against lipid peroxidation, effects on liver lipids, anti-inflammatory activity, antioxidant property and hepatoprotective activity. A brief description of some of these activities is as follows:

2.5.2 Stimulation of Liver Regeneration

One of the mechanisms that can explain the capacity of Silymarin to stimulate liver tissue regeneration is the increase in protein synthesis in the injured liver. In *in vivo* and *in vitro* experiments performed in the liver of rats, silibinin produced a significant increase in the formation of ribosome and DNA synthesis (Magliulo *et al.,* 1973; Sonnenbichler and Zetl, 1986). In rats with experimental hepatitis caused by galactosamine, treatment with intraperitoneal silymarin 140 mg/kg for 4 days completely abolished the inhibitory effect galactosamine on the biosynthesis of liver proteins and glycoproteins.

2.5.3 Anti-oxidant Property

Flavanoids usually possess good antoxidant activity. The water soluble dehydrosuccinate sodium salt of silibinin is a powerful inhibitor of the oxidation of linoleic acid-water emulsion catalysed by Fe^{2+} salts (Ferenci *et al.,* 1989). It also inhibits the microsomal peroxidation produced by NADPH-Fe^{2+} -ADP, a well known experimental system for the formation of hydroxyl radicals (Valenzuela and Guerra, 1986). In studies performed in rat hepatic microsomes, it has been demonstrated that lipid peroxidation produced by Fe (III)/ascorbate is inhibited by silibinin dihemisuccinate; the inhibition is concentration dependant (Bosisio *et al.,* 1992; Mira *et al.,* 1994). The protective effect of silymarin is mediated by the inhibition of lipid peroxidation, and the modulation of hepatocyte Ca^{2+} content seems to play a crucial role (Farghali *et*

al., 2000).

2.5.4 Hepatoprotective Activity

Silymarin is one of the most investigated plant extracts with known mechanisms of action for oral treatment of toxic liver damage (Hiroshi *et al.,* 1984). Silymarin is used as a protective treatment in acute and chronic liver diseases (Flora *et al.,* 1998). Silymarin supports the liver cells through multifactor action including binding to cell membrane to suppress toxin penetration into the hepatic cells, increasing superoxide dismutase activity and glutathione tissue level (Pietrangelo *et al.,* 1995). Also, it inhibits lipid peroxidation (Bosisio *et al.,* 1992; Carini *et al.,* 1992), and enhances hepatocyte protein synthesis (Takahara *et al.,* 1986). The hepatoprotective activity of silymarin can be explained based on antioxidant properties due to the phenolic nature of flavonolignans. It also acts through stimulating liver cells regeneration and cell membrane stabilization to prevent hepatotoxic agents from entering hepatocytes (Fraschini *et al.,* 2002). Recently, it has been shown that flavonolignans inhibit leucotriene production; this inhibition explains their anti-inflammatory and antifibrotic activity (Dehmlow *et al.,* 1996).

2.5.5 Pharmacokinetics

Silymarin is not soluble in water and is usually administered in capsules as a standard extract (70-80% silymarin). Absorption after oral administration is low, with recovery in the bile in rats ranging from 2 to 3%. Peak plasma concentrations are achieved in 4 to 6 hours, both in animals and humans. Silymarin is mainly excreted in the bile and, to a lesser degree, in the urine. Its elimination half-life ranges from 6-8 hours (Morazzoni *et al.,* 1993; Pepping, 1993). However, Janiak *et al*. (1973) reported plasma levels of 500 mg/l (as silibinin) 90 minutes after oral administration 200 mg/kg of silymarin or of purified *S. marianum* extract in mice.

Silibinin and other components of silymarin are rapidly conjugated with sulfate and glucoronic acid in the liver. The conjugates pass into the plasma and to the bile, where they are found in amounts corresponding to 80% of the total dose administered. A negligible portion is eliminated in the urine. These findings suggests the existence of enterohepatic circulation, intestinal absorption, conjugation in the liver, excretion in the bile, hydrolysis by the intestinal flora, and reuptake in the intestine (Mennicke, 1976). The presence of this cycle makes the study of the intestinal absorption of the natural products very difficult. However, the use of labeled silibinin in the rat has made it possible to show that intestinal absorption of a dose of 20 mg/kg amounts to about 35%. Peak radioactivity is found in the plasma 30 minutes after ingestion (Kazazis *et al.*, 2014).

CHAPTER THREE

3.0 Materials and Methods

3.1 Plant Material Collection and Identification

Dried *Phoenix dactylifera L*. (date palm) fruits were obtained from Samaru Market in Zaria, Kaduna, Nigeria, deposited and authenticated in the Herbarium Unit of Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria where the Voucher Specimen Number, 7130, was obtained.

3.2 Experimental Animals

A total of fourty-five (45) Wistar rats of male sex (80 to 125 g) were obtained from the Pharmacology Animal House, Faculty of Pharmaceutical sciences, Ahmadu Bello University Zaria, Kaduna, Nigeria. Animals were housed in wired cages in the Animal House of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, where they were acclimatized for two weeks prior to the commencement of the experiments.

All animals were given rat chow manufactured by Vital Feeds Grand Cereals and Oil Mills Limited, Plateau State, Nigeria; RC 54291, and water allowed *ad libitum*. In addition, the treatment groups were administered aqueous or ethanol fruit extract of *P. dactylifera*/ mercury/ silymarin in addition to water and rat chow. Rats were weighed at the beginning, during and at the end of the study.

3.3 Drugs

- **Mercury**

Seventy grams of mercuric chloride was obtained from Steve Moore chemicals, Emanto, Zaria, and used as the toxin to induce hepato- and nephron-toxicity for the experiment. The product was manufactured by British Drug Houses (BDH) chemicals, Poole, England. Batch number- 291634U.

- **Silymarin**

Silymarin was obtained fron Nana Pharmacy, Rabah road, Kaduna, and used for the experiment as standard antioxidant drug. Each tablet is film coated and contains 140 mg of silymarin. The product was manufactured by Micro Labs Limited 92, Sipcot, Hosur-635 126, India. Batch number- SYFH0011.

3.4 Animal Feed

Pelletized Vital Feed was obtained and used to feed the animals for the experiment. The product was manufactured by Grand Cereals and Oil Mills Limited (GCOML), Km 17, Zawan Roundabout, Plateau State, Nigeria; RC 54291.

3.5 Other Materials

Other materials used in the course of the study were: Digital weighing balance, centrifuging machine, spectrophotometer, Cages, water bottles, and saw dust for bedding, Animal feed and distilled water, Microscope, glass slides, cover slips, stains, fixative (Formal saline) and bottles for fixing tissues, Dissecting set, dissecting boards, orogastric tubes, measuring cylinders, beakers and syringes, Blood sample bottles, tissue paper, gloves and cotton wool.

3.6 Plant Extract Preparation

Preparation of aqueous and ethanol fruit extracts of *P. dactylifera* were conducted in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria.

The method of maceration as reported by Abdul-Wahab *et al.* (2010) and Agbon *et al.* (2013) for the preparation of aqueous and ethanol *P. dactylifera* fruit extract, respectively, were adopted.

The dried *P. dactylifera* fruits were manually separated from the pits and pulverized into powder using laboratory mortar and pestle.

Preparation of the aqueous extract: 250 g of the powder was soaked in 2 litres of cold distilled water in a conical flask. After 24 hours, the mixture of date palm fruit powder and distilled water was filtered using filter rag and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant.

The supernatant was heated (steamed) to dryness in an evaporating dish (Royal Worcester; *Made in England*) using H-H Digital Thermometer Water Bath (Mc Donald Scientific International – 22050Hz1.0A International Number) at 60^{o}C. A yield of 28.20% of the extract was obtained.

Preparation of the ethanol extract: 250 g of the powder was soaked in 1 litre of ethanol in a conical flask. After 24 hours, the mixture of date palm fruit powder and ethanol was filtered using filter rag and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant.

The supernatant was heated (steamed) to dryness in an evaporating dish (Royal Worcester; *Made in England*) using H-H Digital Thermometer Water Bath (Mc Donald Scientific International – 22050Hz1.0A International Number) at 40^{o}C. A yield of 21.28% of the extract was obtained.

3.6.1 *Phoenix dactylifera* Phytochemical Screening

Phytochemical screening of aqueous and ethanol fruit extracts of *P. dactylifera* were done in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria,

Kaduna, Nigeria. The method of Trease and Evans (1983) for Phytochemical screening was adopted.

3.6.2 Dose Preparation of Fruit Extract of *Phoenix dactylifera*

One hundred grams of the extract (aqueous/ ethanol) was measured and dissolved in 800 ml of distilled water. This makes up the working solution. From freshly prepared working solution, little portion was measured out for animal administration.

Different doses of extract were administered to experimental animals by oral gavages using orogastric tube for the experimental period. The method for computing the dosage is shown below:

To determine the equivalent dosage of volume in ml to mg/kg body weight (bw), the following steps were observed:

A) Dosage in mg/kg bw (500 and 1000 mg/kgbw bw of extract)

Taking 1000 mg/kgbw bw for instance, the dosage is calculated as follows:

1000 mg (of extract solute) ----------1 kg (body weight of rat)

1000 mg (of extract solute) ----------1000 g (body weight of rat)

X mg (of extract solute) ----------Y g (measured body weight of rat)

$$\text{X mg/kg (of extract solute)} = \frac{\text{Y g (of rat) x 1000 mg (of extract)}}{\text{1000 g (1 kg bw)}}$$

B) Dosage in ml:

Calculating the dosage in ml equivalent to mg/kg bw, it is important to consider the steps taken in constituting the working solution.

- **Working Solution of Extract:**

100 g (of extract solute) ---------- dissolved in 800 ml (distilled water)

100,000 mg (of extract solute) ---------- dissolved in 800 ml (distilled water)

X mg (of extract calculated from (A) above) ---------- dissolved in X ml

X ml (of extract solution) = $\frac{\text{X mg (of extract) x 800 ml}}{\text{100,000 mg}}$

3.6.3 Dose Preparation of Mercury

15 g of mercuric chloride was dissolved in 300 ml of distilled water to form a solution of mercuric chloride to aid administration of drug easily to the experimental animals by oral gavages. The method for calculating the dosage is as shown below.

To determine the equivalent dosage of volume in ml to mg/kg bw, the following steps were observed:

A) Dosage in mg/kg bw (5 mg/kgbw bw of mercuric chloride)

The dosage is calculated as follows:

5 mg (of toxin solute) ---------- 1 kg (body weight of rat)

5 mg (of toxin solute) ---------- 1000 g (body weight of rat)

X mg (of toxin solute) ----------Y g (measured body weight of rat)

X mg/kg (of toxin solute) = $\frac{\text{Y g (of rat) x 5 mg (of toxin)}}{\text{1000 g (1 kg bw)}}$

B) Dosage in ml:

Calculating the dosage in ml equivalent to mg/kg bw, it is important to consider the steps taken in constituting the working solution.

- **Working Solution of Standard:**

15 g (of toxin solute) ---------- dissolved in 300 ml (distilled water)

15,000 mg (of toxin solute) ---------- dissolved in 300 ml (distilled water)

X mg (of standard *calculated from (A) above*) ----------dissolved in X ml

$$\text{X ml (of toxin solution)} = \frac{\text{X mg (of toxin)} \times 300 \text{ ml}}{15,000 \text{ mg}}$$

3.6.4 Dose Preparation of Silymarin (Standard Drug)

10 g of Silymarin was dissolved in 300 ml of distilled water to form a solution of Silymarin to aid administration of drug easily to the experimental animals by oral gavages. The method for calculating the dosage is as shown below:

To determine the equivalent dosage of volume in ml to mg/kg bw, the following steps were observed:

A) Dosage in mg/kg bw (100 mg/kgbw bw of Silymarin)

The dosage is calculated as follows:

100 mg (of standard solute) ---------- 1 kg (body weight of rat)

100 mg (of standard solute) ---------- 1000 g (body weight of rat)

X mg (of standard solute) ----------Y g (measured body weight of rat)

$$\text{X mg/kg (of standard solute)} = \frac{\text{Y g (of rat)} \times 100 \text{ mg (of standard)}}{1000 \text{ g (1 kg bw)}}$$

B) Dosage in ml:

Calculating the dosage in ml equivalent to mg/kg bw, it is important to consider the steps taken in

constituting the working solution.

- **Working Solution of Standard:**

10 g (of standard solute) ---------- dissolved in 300 ml (distilled water)

10,000 mg (of standard solute) ---------- dissolved in 300 ml (distilled water)

X mg (of standard calculated from (A) above) ----------dissolved in X ml

$$X \text{ ml (of standard solution)} = \frac{X \text{ mg (of stantard)} \times 300 \text{ ml}}{10,000 \text{ mg}}$$

NOTE: The calculated volume (in ml) of the extract/ toxin / standard drug was then administered depending on group and body weight of the experimental animals (Wistar rats).

3.7 Experimental design

Forty five (45) Wistar rats of male sex were divided into nine groups (I-IX) having five rats each. Group I served as control and was administered distilled water at 0.5 ml. Nephrotoxicity and hepatotoxicity were induced in the rats by the administration of mercuric chloride as reported by Sheikh *et al.* (2013).

Group II was administered mercuric chloride at 5 mg/kg body weight (kgbw); 12.5% LD_{50}; 40 mg/kg as reported by Sheikh *et al.* (2013).

Group III was administered Silymarin at 100 mg/kgbw as reported by Ahmed *et al.* (2010), followed by mercuric chloride at 5 mg/kgbw.

Groups IV was administered 500mg/kg of aqueous fruit extract of *P. dactylifera* followed by mercuric chloride at 5 mg/kgbw.

Group V was administered 1000 mg/kgbw of aqueous fruit extract of *P. dactylifera* followed by mercuric chloride at 5 mg/kgbw.

Groups VI was administerd 500 mg/kgbw of ethanol fruit extract of *P. dactylifera* followed by mercuric chloride at 5 mg/kgbw.

Group VII was administered 1000 mg/kgbw of ethanol fruit extract of *P. dactylifera* followed by mercuric chloride at 5 mg/kgbw.

Group VIII was administered 1000 mg/kgbw of aqueous fruit extract of *P. dactylifera* only while, Group IX was administered 1000 mg/kgbw of ethanol fruit extract of *P. dactylifera* only. The administrations were orally done and lasted for a period of 2 weeks as shown in Table 3.1.

Table 3.1: Treatment of Experimental Animals

Group	Treatment/ daily	Duration
I	Distilled water (0.5 ml)	2 weeks
II	Mercury chloride ($HgCl_2$) (5 mg/kgbw)	2 weeks
III	Silymarin (100 mg/kgbw oral) + $HgCl_2$ (5 mg/kgbw)	2 weeks
IV	Aqueous *P. dactylifera* fruit extract (500 mg/kgbw) + $HgCl_2$ (5 mg/kgbw)	2 weeks
V	Aqueous *P. dactylifera* fruit extract (1,000 mg/kg) + $HgCl_2$ (5 mg/kgbw)	2 weeks
VI	Ethanol *P. dactylifera* fruit extract (500 mg/kgbw) + $HgCl_2$ (5 mg/kgbw)	2 weeks
VII	Ethanol *P. dactylifera* fruit extract (1000 mg/kgbw) + $HgCl_2$ (5mg/kg)	2 weeks
VIII	Aqueous *P. dactylifera* fruit extract (1000 mg/kgbw) only	2 weeks
IX	Ethanol *P. dactylifera* fruit extract (1000 mg/kgbw) only	2 weeks

Note: All administrations were via oral route.

3.8 Experimental Procedures

All experimental procedures were carried out in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, except otherwise specified.

3.8.1 Animal Sacrifice

At the end of the experiment, Wistar rats were humanely sacrificed under chloroform anesthesia. The rats were dissected, blood samples were collected with the use of sample bottles and the organs of interest, liver and kidney were excised, weighed and fixed in 10% formal saline.

3.8.2 Weight Change

The initial (first day of administration) and final (last day of administration) body weights of animals in all the groups were recorded and analysed to determine the weight change between the various groups. The organ weight / body weight ratio of animals in all the groups were also recorded and analysed.

3.8.3 Biochemical Studies

Biochemical studies were carried out using the collected blood samples in the Department of Chemical Pathology, Faculty of Medicine, Ahmadu Bello University Teaching Hospital, Shika. Biochemical analysis for liver serum markers namely Alanine transaminase-ALT, Alkaline phosphstase- ALP and Aspartate aminotransferase- AST; kidney electrolytes namely Na^+, K^+, Cl^-, HCo_3^- and Urea and lipid peroxide levels and antioxidant enzyme activity namely malondialdehyde - MDA, superoxide dismutase - SOD, catalase - CAT and glutathione peroxidase - GSHPx were performed.

The principles involved in performing these biochemical analyses are enumerated below.

Liver Serum Markers

ALP

Reagents

Alkaline phosphatase reagent obtained from Pointe Scientific, Inc. Reconstituted reagent with 15 mL distilled water was gently swirled to dissolve. The reconstituted reagent contained p-Nitrophenylphosphate 10.0 mM, Magnesium ions 1.0 mM, Buffer (pH 10) , activator and binder. The un reconstituted reagent was stored at 2-8°C. Once reconstituted, the reagent was stable for 48 hours at 25°C and for 30 days at 2-8°C.

Materials

Spectrophotometer, Spectrophotometer cuvettes, Distilled deionized water, Physiological (0.9% NaCl) saline, Pipettes, Paraffin squares, Heating block or water bath 37°C and Timer.

Procedure

The spectrophotometer was turned on and allowed to warm up for at least 15 minutes. The wavelength was set to 405 nm. Cuvettes were labelled. 1.0 mL of distilled deionized water was added to cuvette 1. 1.0 mL of Alkaline phosphatase reagent was added to each control and patient cuvettes. All cuvettes were incubated at 37°C for 5 minutes. 25 uL of each control and patient sample was added to their respective cuvettes. This was mixed by inversion using the paraffin squares to prevent spillage. Control and patient cuvettes were incubated at 37°C for 1 minute. After one minute, cuvette 1 was placed in the spectrophotometer and set the absorbance to read 0.000. The absorbance for each of the control and patient cuvettes were recorded. The control and patient cuvettes were returned to 37°C and readings were repeated for each cuvette everyminute for the next two minutes. The average absorbance difference per minute was calculated. (ΔAbs/min).

Calculation

The amount of enzyme was calculated using the following:

$$\text{ALP (IU/L)} = \frac{\Delta\text{Abs/min} \times \text{TV} \times 1000}{18.75 \times \text{LP} \times \text{SV}}$$

Where ΔAbs/min	=	Average absorbance change per minute
1000	=	Conversion of IU/ml to IU/L
1.025	=	(TV) Total reaction volume (mL)
18.75	=	Millimolar absorptivity of ρ-Nitrophenol
.025	=	(SV) Sample volume (mL)
1	=	(LP) Light path in cm

AST

Reagents

AST Substrate: 33 mM Aspartic acid 5 mM ketoglutaric acid phosphate buffer pH 7.4. AST Color Reagent: 0.25% w/v Diazonium salt preserved with formalin. AST Calibrator: A lyophilized serum with AST value provided in each lot Reconstituted with distilled water was allowed to stand until it dissolved.

Materials

Blood Serum, Pipetting devices, Test tubes/rack, Timing device, Spectrophotometer, Heating block or bath (37°C) and 0.1 N Hydrochloric Acid.

Procedure

0.5 ml of AST substrate was placed into test tubes labeled "Blank" "Calibrator" "Control" and

"Unknowns". Vials were warmed in 37°C heating bath for at least four (4) minutes. 0.1 ml (100µl) of samples were added at timed intervals into their respective tubes and were mixed gently and returned to 37°C heating bath for exactly ten (10) minutes (Distilled water was used for sample blank). 0.5 ml of AST (SGOT) color reagent was added after ten (10) minutes and in the same timed sequence. This was mixed gently and immediately returned to 37°C heating bath for another ten (10) minutes.

2.0 ml of 0.1 N Hydrochloric acids was added after ten (10) minutes add and mixed by inversion. The wavelength of the spectrophotometer was set at 530 nm and zeroed. The absorbance of all tubes were recorded.. (Wavlength range: 500 - 550)

NOTE: The final color developed in the reaction was read within sixty (60) minutes.

Calculation

Use the absorbance reading of the calibrator and specimen provided to calculate AST

$$\frac{\text{Abs. of unknown}}{\text{Abs. of calibrator}} \text{ x AST concentration of Calibrator IU/L = AST value}$$

ALT

Reagents

ALT substrate: 0.2 M L-alanine 2.0 mM a-ketoglu- tarate 100 mM phosphate buffer at pH 7.4 + 0.05 0.2% v/v preservatives.

ALT color reagent: l.0mM 2 4-dinitrophenylhydrazine in 1N Hydrochloric Acid preservative.

ALT Color developer: 0.5N sodium hydroxide.

ALT calibrator: Solution of sodium pyruvate in 100 mM phosphate buffer at pH 7.4 . The activity will be provided in each lot.

Materials

Blood serum, Pipetting devices, Test tubes/rack, Timer, Spectrophotometer with capability to read at 505 nm and Heating block or bath (37°C).

Procedure

Test tubes were labelled accordingly and 0.5 ml of ALT substrate was added to each tube and placed in a 37° Cheating bath for 3-5 minutes. At timed intervals (about 15-30 seconds) 0.1 ml (100µl) of sample was added to the correspondingly labeled tube, mixed and immediately returned to 37°C healing bath for exactly 30 minutes. After exactly 30 minutes 0.5 ml of ALT Color Reagent was added to each tube maintaining the timed interval sequence which was also mixed and returned to 37°C heating bath for exactly 10 minutes. 2.0 ml of ALT Color Developer (maintaining the same timed intervals) was then added, mixed and returned to 37°C heating bath for five (5) minutes. The spectrophotometer was zeroed with the reagent at 505 nm. Absorbance of all tubes were recorded.

Calculation

Use the absorbance reading of the calibrator and unknown(s) to calculate ALT.

$$\frac{\text{Abs. of specimen}}{\text{Abs. of Calibrator of specimen (IU/L)}} \times \text{conc. of Calibrator (IU/L)} = \text{ALT values}$$

Kidney Electrolytes

Na^+

Reagents

Sodium Color Reagent, Solution of uranyl acetate, 5.3 g/dL, and zinc acetate, 15.4 g/dL, in aqueous acetic acid-ethanol mixture. Precipitating Reagent, Aqueous solution of trichloroacetic acid (TCA), 10 g/dL. Sodium Standard - (140 mmol/L), Sodium chloride, 4.091 g/L in aqueous TCA, equivalent to sodium value of 140 mmol/L when used as directed in method presented.

Materials

Spectrophotometer capable of absorbance readings at 420 nm, Centrifuge with high speed capacity (>1500 rpm), Pipets capable of accurately delivering 0.5 and 2.5 mL, Test tubes & Cuvets Vortex mixer (optional), Interval timer.

Procedure

Preparation of Protein-Free Supernate

0.5 mL of serum plasma was added to properly labeled test tubes (Do not use Standard in this step!)

0.5 mL Precipitating Reagent dropwise was added to each tube with vigorous mixing (vortexing suggested) and allowed to stand for 5 minutes, then centrifuged at high speed for 5-10 minutes.

Test Procedure

The following volumes (mL) were pipetted into marked tubes and mixed promptly after each color reagent was added:

Reagent	Standard (S)	Sample (U)
Distilled		

Water	0.5		
Standard		0.5	
Supernate			0.5
Color Reagent	2.5	2.5	2.5

Contents of all tubes were again remixed and Incubated for 10 minutes at room temperature (15-30oC). After the incubation period, the test tubes were centrifuged at high speed for 5 minutes. The supernate was Carefully transferred to appropriate cuvet. With the spectrophotometer set at 420 nm, the instrument was zeroed with water. Absorbance of the Reagent Blank (RB), Standard (S) and Unknowns (U) within 30 minutes was recorded.

K^+

Reagents
Potassium reagent: sodium tetraphenylboron 2.1 mM, preservatives and thickening agents.

Potassium standard: Equivalent to 4 mEq/L.

Materials
Spectrophotometer, Test tubes, Timer.

Procedure
Test tubes were labeled and 1.0 ml of potassium reagent was pipetted into all tubes, 0.01 ml samples were added to respective tubes. This was mixed and allowed to sit at room temperature for 3 minutes, then the spectrophotometer was set to 500 nm and zeroed with reagent blank. Absorbance of all tubes was recorded.

Calculations

Abs. = absorbance

STD = standard

$$\frac{\text{Abs. of unknown}}{\text{Abs. of STD}} \times \text{Conc. Of STD (mEq/L)} = \text{potassium Conc. (mEq/L)}$$

Cl^-

Reagents

Chloride reagent

Chloride calibrator: sodium chloride 100 mEq/L

Materials

Test tubes, Timer, Accurate pipetting devices, Spectrophotometer able to read at 460 – 550nm.

Procedure

Test tubes were labelled; 1.5 ml chloride reagent was added to each tube. 0.01 ml of calibrator or sample was added to the tubes and mixed. The mixture was incubated at room temperature for atleast five minutes, spectrophotometer was set to 480 nm and zeroed with reagent blank. Absorbance reading of all tubes were recorded.

Calculation

$$\frac{\text{Abs. of unknown}}{\text{Abs. of calibrator}} \times \text{Conc. of calibrator} = \text{Conc. of chloride (mEq/L).}$$

Urea

Reagents

Working reagent concentrations: Urease (Jack Bean) >15,000 U/L, GLDH (Bovine) >200 U/L, ADP >0.6 mM, α-Ketoglutarate 3.6 mM, NADH >0.28 mM, Buffer, pH 7.8 ± 0.1, stabilizers, Sodium Azide (0.25%) as preservative.

Materials

Urea Nitrogen Enzyme Reagent (R1), Urea Nitrogen Coenzyme Reagent (R2), Accurate pipetting devices. (10ul and 1.0ml), Timer. (Able to measure 30 and 60 second intervals), Test tubes/rack, Spectrophotometer with a temperature controlled cuvette able to measure at 340nm.

Procedure

Working reagent was prepared according to instructions. Spectrophotometer was zeroed with water at 340 nm. 1.0ml of reagent was added into test tubes and allowed to come to 37°C. 0.01ml (10ul) of calibrator, control, or sample was added to test tubes and immediately placed in the spectrophotometer. After thirty seconds the absorbance was recorded (A1). Sixty seconds after the first reading, another reading was recorded (A2). The absorbance change between the two readings (A1-A2) was determined. Procedure was repeated for each sample.

Calculation

(A1-A2) = Absorbance change between readings

(A1 – A2) unknown x concentration = BUN (mg/dl)

(A1 – A2) standard of standard

Creatinine

Equipments

Spectrophotometer, Spectrophotometer cuvettes, 5 mL test tubes, Pipettes, Paraffin squares, Timer, Distilled deionized water, Picric acid, 0.036 mol/L.

Reagents

Dissolve 9.16 g reagent grade picric acid in warm water, cool and make up to 1 L volume. Tungstic Acid in Polyvinyl Alcohol. Place 1 g polyvinyl alcohol in 100 mL water and heat to dissolve but do not boil. Transfer to a 1 L volumetric flask containing 11.1 g $Na_2WO_4 \cdot 2H_20$ in 300 mL of water. Then add 2.1 mL concentrated H_2SO_4 in 300 mL water. Mix, and dilute with water to 1 L volume. This solution is stable at room temperature (25°C) for 2 years and does not require refrigeration. NaOH, 1.4 mol/L. Dissolve 54 g NaOH in water and dilute to 1 L volume. Store in a polyethylene bottle. Creatinine standards, 0.5 mg/dL, 1.0 mg/dL, 2.0 mg/dL, 4.0 mg/dL, 10.0 mg/Dl.

Procedure

The spectrophotometer was turned on and allowed to warm up for at least 15 minutes. The wavelength was set to 520 nm. Urine specimens were diluted with distilled deionized water at ratio 1:10. The proteins present in the patient serum or urine specimens were precipitated. This was mixed vigorously and centrifuged for 10 minutes. Cuvettes were labelled 1 through 10 and 1.0 mL of picric acid solution was added to cuvettes 1 through 10. 3.0 mL of distilled deionized water was added to cuvette 1. 3.0 mL of the 0.5 mg/dL creatinine standard was added to cuvette 2. 3.0 mL of the 1.0 mg/dL creatinine standard was added to cuvette 3. 3.0 mL of the 2.0 mg/dL creatinine standard was added to cuvette 4. 3.0 mL of the 4.0 mg/dL creatinine standard was added to cuvette 5. 3.0 mL of the 10.0 mg/dL creatinine standard was added to cuvette 6. 3.0 mL of the protein free centrifugate of control Level One was added to cuvette 7. 3.0 mL of the protein free centrifugate of control Level two was to cuvette 8. 3.0 mL of the patient's protein free serum or urine centrifugate was added to the remaining cuvettes. Mixed by inversion using a

paraffin square to prevent spillage. 0.5 mL of the NaOH solution was added to the first cuvette. The timer was set for 15 minutes. 0.5 mL of the NaOH was added to the remaining cuvettes at 30 second intervals. After 15 minutes, cuvette 1 was placed in the spectrophotometer and the Absorbance was set to read 0.000. Absorbance was read at exactly 15 minutes after adding the NaOH and recorded.

Lipid Peroxidation and Antioxidant Enzyme Activity

MDA

Estimation of lipid peroxidation was assayed spectrophotometrically using thiobarbituric acid-malondialdehyde assay (TBA-MDA). Lipid peroxides were extracted using 0.5 g fresh tissue with 0.3 ml of 1% meta-phosphoric acid (pH 2) and 1 ml of 0.6% thiobarbituric acid (TBA) in air tight tube and kept in a boiling water bath for 45 min. The samples were cooled at room temperature and 5 ml butanol was added along with stirring of mixture. The butanol phase was separated by centrifugation at 1000 rpm for 10 min and transferred to glass cuvettes. The color of TBA chromogen was measured at 532 nm.

$$\text{MDA level} = \frac{[OD532 \times 100/1.56] \times TV}{[d\ wt \times 1000]}$$

OD = optical density; TV = total volume of the extract (ml); d wt = weight of the dry leaf tissue (g)

SOD

Reagents

- 0.067 M Potassium phosphate buffer, pH 7.8
- 0.1 M Ethylene diamine tetraacetic acid (EDTA) containing 0.3 mM sodium cyanide
- 0.12 mM Riboflavin (store cold in a dark bottle)

- 1.5 mM Nitroblue tetrazolium (NBT) (store cold)

Procedure

Pipette into a series of tubes:

EDTA/Cyanide	0.2 ml
NBT	0.1 ml
Enzyme	*
Phosphate buffer	q.s. to 3.0 ml

Include several tubes with no enzyme as controls.

A series of samples ranging from 0.1-10 micrograms is recommended. A tube containing approximately 100 micrograms will generally produce maximum inhibition. The tubes were placed in a light box which provided uniform light intensity. The tubes were incubated for 5-8 minutes to achieve a standard temperature. At zeroed time and at timed intervals, 0.05 ml riboflavin was added. All tubes in the light box were incubated for 12 minutes and at timed intervals read A_{560}. Percentage inhibition of NBT reduction was determined and Plotted against amount of enzyme in test. The amount of enzyme resulting in one half of maximum inhibition was determined.

Calculation

$$\text{Units/mg} = \frac{1000}{\quad\quad}$$

ug enzyme resulting in half max. inhibition

CAT Procedure

10ul of serum was added to a test tube containing 2.8ml of 50mM potassium phosphate (buffer pH 7.0). The reaction was initiated by adding 1.0ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041mm was used to calculate the CAT activity.

$$\frac{\text{Absorbance of substance}}{E} \times \text{Protein concentration (mg/ml)}$$

GPx

Reagents

0.2 M phosphate buffer; 10g of Trichloro-acetic acid (TCA), distilled water and Ellman's reagent comprising of 19.8mg of 5,5'- dithiobis (nitro benzoic) in 100ml of 0.1% sodium nitrate.

Procedure

1.5 ml of 10% TCA was added to 150ul of serum in phosphate saline at pH of 7.4 and centrifuged at 4000rpm for 5 mins. 1ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3ml of phosphate buffer (pH 8.0) the absorbance was read at 412nm.

The quality of GPx was derived from the graph of the GPx standard curve.

3.8.4 Histological and Histochemical Studies

At the end of the experiment, harvested organs (liver and kidney) were fixed in 10% formal saline and tissues were processed for routine histological examination, stained with Haematoxylin and Eosin (H&E). Histochemical staining (Periodic Acid Schiff for demonstrating glycogen and Godon and Sweet for demonstrating reticular fibres) were done and examined under the light microscope. Tissue preparation, stain preparation and staining protocols are as follows:

3.8.5 Tissue Processing

Harvested organs were fixed in 10% formal saline to prevent degradation and to maintain the structure of cellular components and tissues processed for routine histological and histochemical examination. The main constituent of biological tissue, water, was removed in the process of dehydration. Samples were transferred through baths of progressively more concentrated (65% - 100%) ethanol to remove the water. This was followed by a hydrophobic clearing agent such as xylene used to remove the alcohol, and finally molten paraffin wax, the infiltration agent, was used to replace the xylene. The tissue samples were then placed into molds along with liquid embedding material (wax) which was then hardened. This was achieved by cooling the paraffin wax. The hardened blocks containing the tissue samples were then ready to be sectioned. A steel knife mounted on a microtome was used to cut the tissue samples to produce series of thin sections of precise thickness (5-10um) which were mounted on a glass slide.

3.8.6 Preparation of Staining Solutions and Staining

Haematoxylin and Eosin Stains

Heamatoxylin was dissolvded in alcohol and alum which was dissolved in water was added. The mixture was then boiled and mercuric acid added when solution turn dark purple.it was then cooled rapidly.

5% eosin solution was made `by dissolving 5 g of eosin in 100 ml of distilled water. To prevent growth of mould in the stock solution, a crystal of thymol was added to obtain 1% staining solution, 20 ml of the stock solution was diluted by making it up to 100ml.

H and E Staining

The tissues are cleared with a clearing agent and rehydrated using decreasing concentration of ethanol (100% - 65%). The tissues were then stained with haematoxylin (which stains negatively charged components such as DNA) and eosin (used as a counter stain for the cytoplasm). The tissues are then examined under the light microscope.

Preparation of PAS Stain

0.5g of periodic acid was added to 100ml of distilled water which was properly mixed and labelled.

PAS Staining

The tissue samples were deparaffinized and hydrated and then oxidize in 0.5% periodic acid solution for 5 minutes, the samples were then rinsed in distilled water and then Placed in Schiff reagent for 15 minutes (Sections became light pink color during this step), the sections were washed in lukewarm tap water for 5 minutes (Immediately sections turned dark pink color). The sections were then counterstained in Mayer's hematoxylin for 1 minute and then washed in tap water for 5 minutes after which they

were dehydrated and cover slipped using a synthetic mounting medium. A red purple colour indicated the presence glycogen.

Preparation of Silver Stain

Solution

5 mL of the 10% silver nitrate was placed in a 100 mL flask. Using a pasteur pipette, a drop of strong ammonium hydroxide was added and then swirled for a few seconds. A precipitate was formed at first. Ammonium hydroxide was continuously added drop by drop and swirld until the precipitate just redissolved. 5 mL of 3% sodium hydroxidewas added and mixed. A precipitate was again formed. Drops of ammonium hydroxide were added until the precipitate just redissolved, leaving a faint opalescence which was diluted to 50 mL with distilled water.

Gordon and Sweet Staining

The tissue samples were de-paraffinised and hydrated with distilled water and placed in acidified potassium permanganate for 10 minutes after which they were rinsed off in distilled water and placed in Oxalic acid for 3 minutes, after which they were again rinsed in distilled water. Samples were then transfered to Iron alum for 15 minutes and rinsed with distilled water 3-4 times to cut down the background staining. They were then placed in ammoniacal silver for 70 seconds (for best result each slide was stained separately). The silver solution was poured on, tipped off and re-applied all in one action to ensure even staining of the sections and then washed 3 times for 10 seconds each in distilled water and then added developer till it turns golden. It was then washed well in water and then Fixed in sodium thiosulphate (hypo) for 3 minutes. It was washed well again in water then counter stained in

0.1% neutral red for a minute, then dehydrated, cleared with a clearing agent and mounted.

3.8.7 Data Analysis

Results obtained were analyzed using Statistical Package for Social Sciences (SPSS *version 18.0*) and results were expressed as mean ± S.E.M. The presence of significant differences among means of the groups were determined using one way Analysis of variance (ANOVA) with Least significant difference (LSD) for significance. Paired sample *t*–test was employed for the comparisons of means as appropriate. Values were considered significant when $p \leq 0.05$.

CHAPTER FOUR

4.0 Results

4.1 Phytochemical Analysis

Phytochemical analysis of aqueous and ethanol fruit extracts of *Phoenix dactylifera* indicating the presence of secondary metabolites is as shown in **Table 4.1**.

4.2 Physical Observation

The physical activities of experimental animals (Wistar rats) were observed during the period of administration. Wistar rats in the Control Group were observed to exhibit normal physical activities, such as movement and playfulness, whereas animals in the treatment Groups exhibited decreased activities such as sluggishness and loss of appetite especially in mercury ($HgCl_2$) treated Group.

4.2.1 Weight Change

The weights of the Wistar rats, in all Groups (I - IX), were observed when the initial and final weights were compared. The result showed that weight increase was observed in all treated Groups. The result also showed that weight change in all treated Groups were significantly lower when compared to the Control, except in Group VII ($p>0.05$). Weight change in Group VII was significantly higher when compared to Group II ($p<0.05$) (**Table 4.2**).

Table 4.1: Phytochemical Constituents of the Fruit Extracts of *Phoenix dactylifera*

Constituents	Aqueous extract	Ethanol extract
	Inference	Inference
Carbohydrates	+	+
Anthraquinones	–	–
Cardiac glycosides	–	–
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	+

+ = Positive (present)
– = Negative (absent)

Table 4.2: Weight change and weight comparison of Wistar rats *first* day and *final* day of treatment

Group/ Treatment	IW (g)	FW (g)	FW-IW (g))	*t*	p-value
I	125.50±12.34	174.45±39.65	48.95±27.31	1.268	0.425
II	93.10±3.02	117.07±4.16	23.97±1.14*	2.467	0.090
III	81.28±5.51	103.45±4.85	22.17±0.66*	2.569	0.083
IV	102.40±8.78	134.90±9.52	32..50±0.74*	4.339	0.012
V	97.18±6.98	120.72±5.78	23.54±1.12*	2.933	0.043
VI	86.48±4.45	114.33±4.11	27.85±0.34*	17.472	<0.001
VII	99.48±2.68	122.40±4.30	22.92±1.62*	2.145	0.278
VIII	80.26±3.49	102.10±5.73	21.84±2.24*	9.258	0.001
IX	95.06±5.34	123.70±4.87	28.64±0.47*	12.518	<0.001

n = 5; mean ± SEM; Paired sample *t*- test;*****= $p<0.05$, *****= $p<0.001$; significant difference when compared with the *control*. **Gp I**=Control (distilled H_2O 0.5 ml);**II**= $HgCl_2$ (5 mg/kg); **III**= Silymarin **(**100mg/kg);**IV and V=** *Aqueous fruit extract of P.dactylifera* (500 and 1,000 mg/kg respectively);**VI and VII=** *Ethanol fruit extract of P.dactylifera* (500 and 1,000 mg/kg respectively)*;aqueous fruit extract of P.dactylifera* (1,000 mg/kg);*Ethanol fruit extract of P.dactylifera* (1,000 mg/kg)**FW**=Final weight; **IW**= Initial weight; **FW-IW**= Weight change.

4.2.2 Organ Weight / Body Weight Ratio

The organ (liver) weight and the body weight (BW) of Wistar rats were compared and the results revealed that the liver/ body weight index was significantly decreased in Groups I, II and IX when compared to Control ($p<0.05$). The Liver/ body weight index was significantly increased in Groups V, VI and VII when compared to Group III ($p<0.05$) (**Figure 4.1**).

The organ (kidney) weight and the body weight (BW) of Wistar rats were compared and the results revealed that the kidney/ body weight index was significantly decreased in Groups II, III, IV, V, VIII and IX when compared to Control ($p<0.05$). The results also revealed that the kidney- body weight index was significantly increased in Groups VI and VII when compared to Group II ($p<0.05$) (**Figure 4.2**).

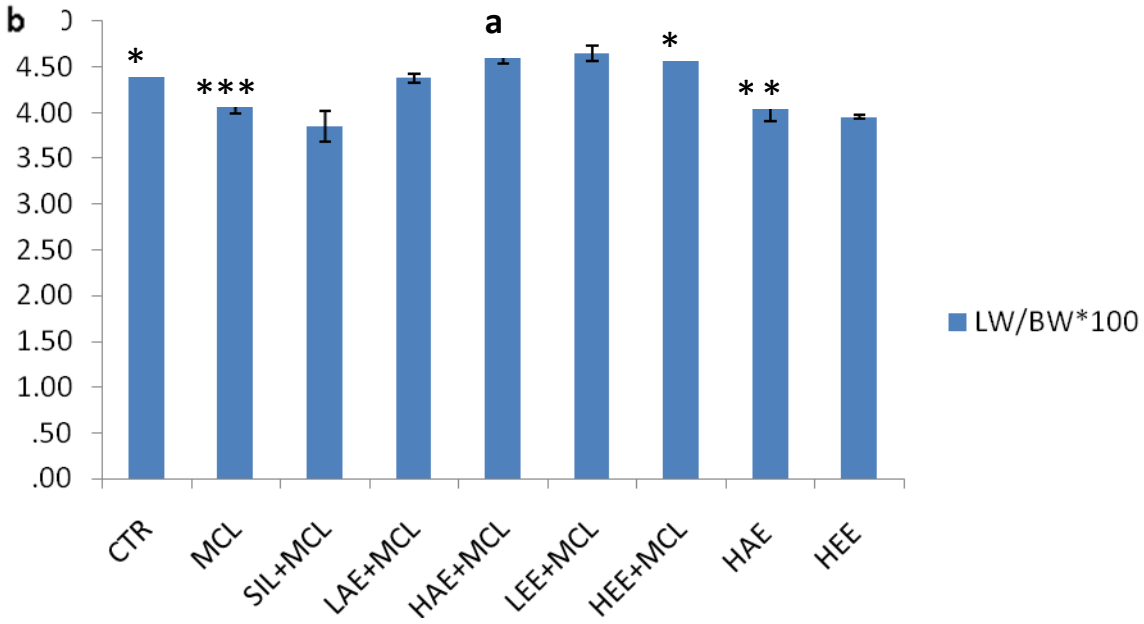


Figure 4.1: Comparison of organ (liver) - body weight index of Wistar rats

n = 5; mean ± SEM; Paired sample *t*- test; One way ANOVA *LSD post hoc test:* *****= p <0.05, ******= p <0.01, *******= p <0.001; significant difference when compared with the *control*; ***a*** = p <0.05, ***b*** = p <0.01; significant difference when compared with the MCL group. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg); **LW**=Liver weight; **BW**= Body weight.

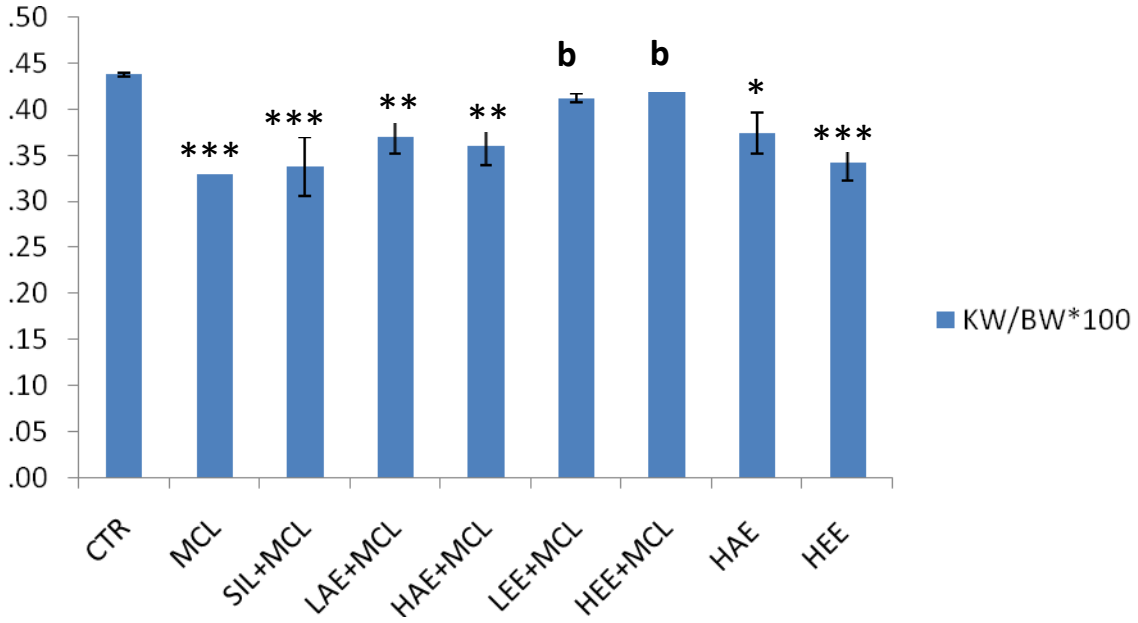


Figure 4.2: Comparison of organ (kidney) - body weight index of Wistar rats

n = 5; mean ± SEM; Paired sample *t*- test; One way ANOVA *LSD post hoc test:* *****= p <0.05, ******= p <0.01, *******= p <0.001; significant difference when compared with the *control*; ***b*** = p <0.0; significant difference when compared with the MCL group. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg); **KW**=Kidney weight; **BW**= Body weight.

4.4 Biochemical Studies

Biochemical studies involved the analyses of serum liver enzymes, kidney electrolytes, lipid peroxidation and antioxidant enzyme activities of the experimental animals (Wistar rats).

4.4.1 Serum Liver Enzymes

Analysis of serum liver enzymes of Wistar rats revealed elevation of serum liver enzymes; aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels in the treatment groups. The results of the AST showed increased levels of AST in all the treated Groups with significant increase in Group VIII when compared to the Control Group ($p<0.05$) as shown in Figure 4.3. The results of the ALT showed non significant increased levels of ALT in all the treated Groups when compared to the Control Group ($p>0.05$) except Group VI which showed non significant decreased ALT levels ($p>0.05$) as shown in Figure 4.4, while the results of ALP analysis showed significant increased levels of ALP in Groups II, IV, VI and IX when compared to the Control Group ($p<0.05$) as shown in Figure 4.5.

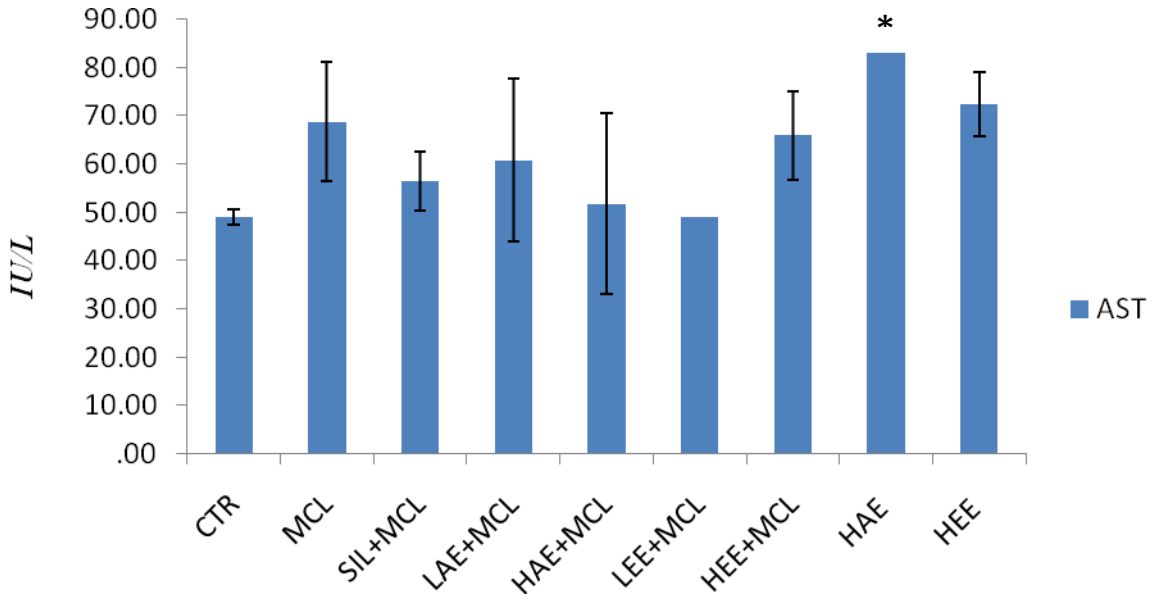


Figure 4.3: Effect of fruit extracts of *P. dactylifera* on Aspartate Transaminase (AST) against mercury-induced liver toxicity in Wistar rats

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test:* ***** = p <0.05; significant difference when compared with the *control*. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

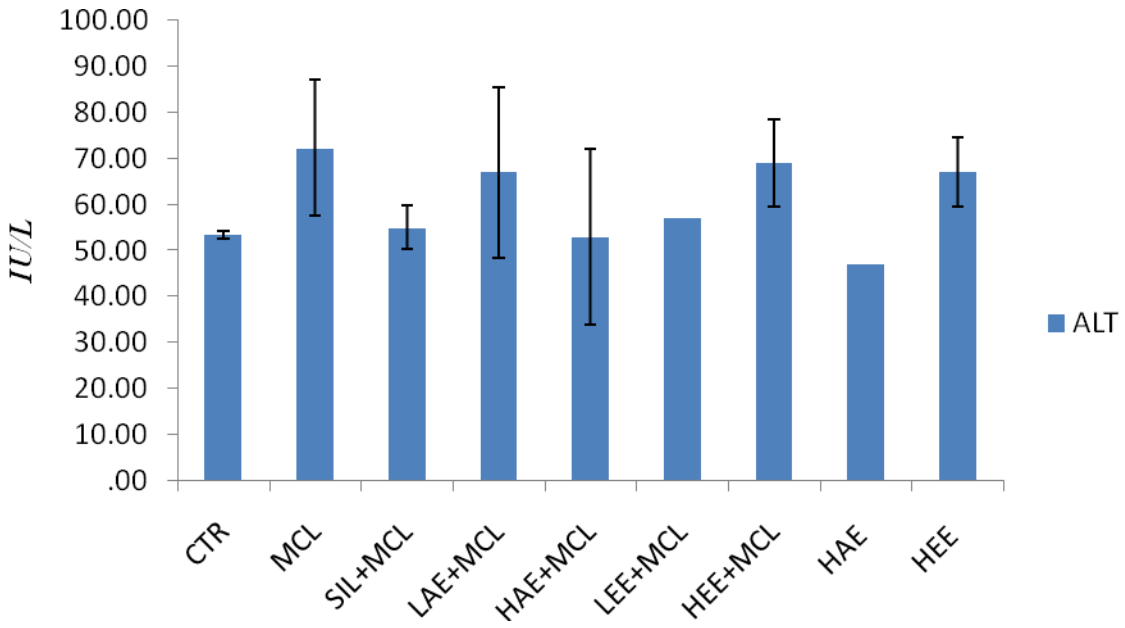


Figure 4.4: Effect of fruit extracts of *P. dactylifera* on Alanine Transaminase (ALT) against mercury-induced liver toxicity in Wistar rats

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test, p*> 0.05; when compared with the *control*. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

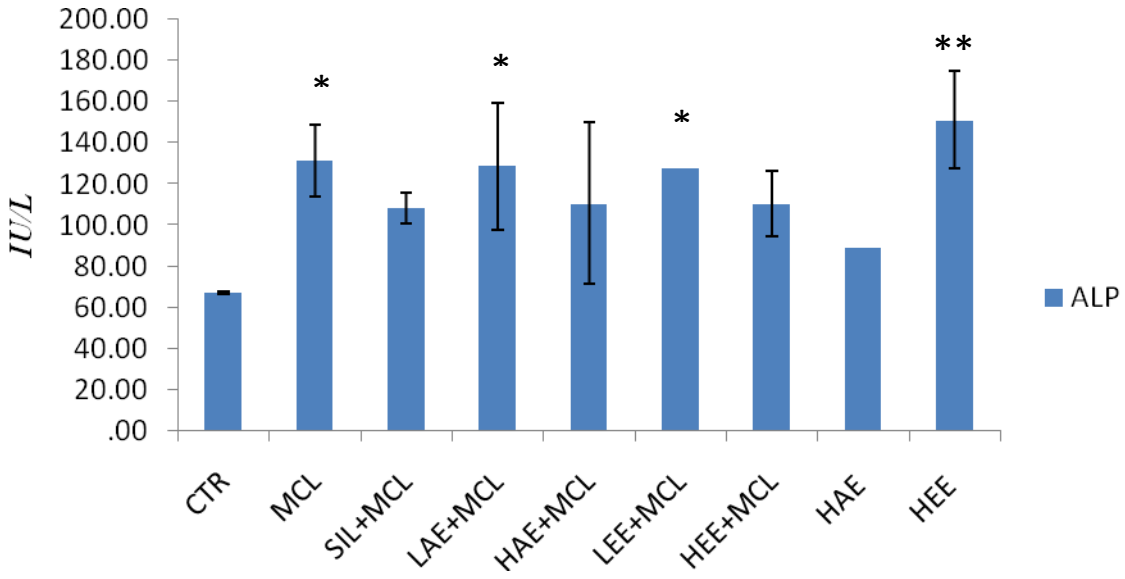


Figure 4.5: Effect of fruit extracts of *P. dactylifera* on Alkaline Phosphatase (ALP) against mercury-induced liver toxicity in Wistar rats

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test:* ***** = p <0.05; ****** = p <0.01; significant difference when compared with the *control*. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

4.4.2 Kidney Electrolytes

In the present study, serum kidney electrolytes (urea, sodium ion (Na^+), chloride ion (Cl^-), potassium ion (K^+), bicarbonate (HCO_3^-)) and creatinine levels of experimental animals (Wistar rats) were analysed. The results of serum urea showed non significant decrease in urea levels in all the treatment Groups as compared to the Control Group ($p>0.05$) except Groups II, V and VIII that showed non significant increase in urea levels ($p>0.05$) as shown in Table 4.3.

The results of creatinine showed non significant increased levels of creatinine in all treated Groups as compared to Control Group ($p>0.05$) except Group VII which showed non significant decreased creatinine level, while Groups VI and VIII showed significantly decreased creatinine levels ($p<0.05$). The results of creatinine also showed non significant decreased creatinine levels in all the treated Groups when compared to Group II ($p>0.05$) with significant decrease observed in Group VII ($p<0.05$) as shown in Table 4.3.

The Results of Na^+ analysis showed non significant increase in Na^+ levels in all the treatment Groups when compared to the Control Group except Groups IV and VII that showed non significant decrease in Na^+ levels ($p>0.05$) and significantly decreased Na^+ level in Group VIII ($p<0.05$). Na^+ analysis also showed significantly decreased levels in Groups VI and VIII as compared to Group II ($p<0.05$) as shown in Table 4.3.

When compared to the Control Group, the results of Cl^- showed non significant increase in Cl^- levels in Groups II, III and VII ($p>0.05$), significantly increased Cl^- level in Group IX ($p<0.05$), non significant decrease in Cl^- levels in Groups IV, V, VI and significantly decreased Cl^- level in Group VIII ($p<0.05$) as shown in Table 4.3.

The results of K^+ showed non significant decrease in K^+ levels in all the treated Groups with significant

decrease in Group IX as compared to the Control Group ($p<0.05$) except Groups VII and VIII where non significant increase in K^+ levels were observed ($p>0.05$). Non significant Increase in K^+ levels were shown in all the treatment Groups as compared to Group II ($p>0.05$) except Groups VI and IX that showed non significant decreased K^+ levels as shown in Table 4.3.

The results of HCO_3^- showed non significant increase in HCO_3^- levels in all the treated Groups as compared to the Control Group ($p>0.05$) except Groups VI and VIII that showed significantly decreased HCO_3^- levels ($p<0.05$). When compared to Group II, non significant decrease in HCO_3^- levels were shown in all the treatment Groups except Groups IV, V and IX that showed non significant increased HCO_3^- levels ($p>0.05$) as shown in Table 4.3.

Table 4.3: Effect of fruit extracts of *P. dactylifera* on kidney electrolytes against mercury-induced kidney toxicity in Wistar rats

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test:* *****= p <0.05, ******= p <0.01, *******= p <0.001; significant difference when compared with the *control*; ***b*** = p <0.01, ***c*** = p <0.001; significant difference when compared with the MCL group. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

4.4.3 Lipid Peroxide Levels and Antioxidant Enzyme Activity

The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assay was used to estimate lipid peroxidation levels which is a marker of lipid peroxidation, while antioxidant enzymatic activity was estimated by assaying for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in the serum of Wistar rats.

Serum MDA levels were increased significantly in Group II when compared to the Control Group ($p<0.05$). Groups VI, VII and VIII showed non significant increase in MDA levels, while non significant decrease in MDA levels were observed in Groups III, IV and V when compared to the Control ($p>0.05$). When compared to Group II, MDA levels were decreased significantly in all the treated Groups ($p<0.05$) as shown in Figures 4.6.

The serum SOD results showed non significant increased SOD activity in Groups III, V, VI, VII, IX ($p>0.05$) and significantly increased SOD activity in Group IV as compared to the Control Group ($p<0.05$). Groups II and VIII showed non significant decreased SOD activity as compared to the Control. Significantly increased SOD activity was observed in Group IV as compared to Group II ($p<0.05$) while all other treatment Groups showed non significant increase in SOD activity except Group VIII that showed non significant decrease in SOD activity as shown in Table 4.7.

Analysis of serum CAT activity revealed non significant increase in CAT activity in all the treatment Groups when compared to Control ($p>0.05$), except Groups II and VIII that showed non significant decrease in CAT activity. When compared to Group II, Group VIII revealed non significant decrease in CAT activity, while all other treated Groups showed non significant increase in CAT activity as shown in Table 4.8.

Serum GPx activities were non significantly increased in all treated groups except Group II, VI and VIII when compared to the Control Group ($p>0.05$). Non significant decrease in serum GPx activities were observed in all treated Groups, except Groups VI and VIII when compared to Group II as shown in Figures 4.9.

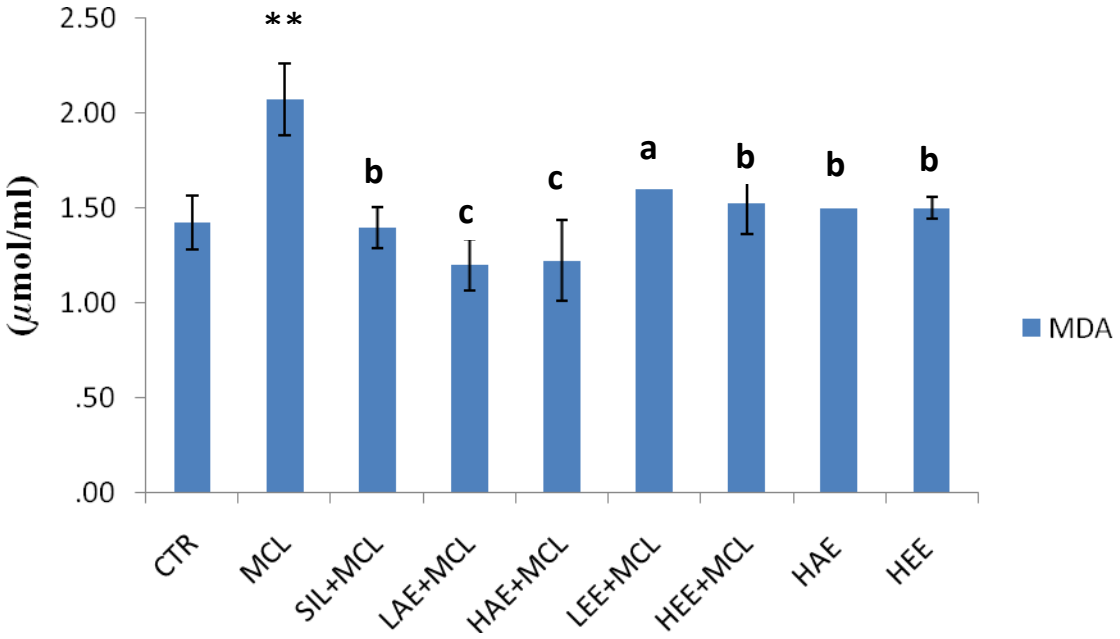


Figure 4.6: **Effect of fruit extracts of *P. dactylifera* on blood serum Malondialdehyde (MDA) concentration of Wistar rats**

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test:* ****** = p <0.01; significant difference when compared with the *control*; ***a*** = p <0.05, ***b*** = p <0.01, ***c*** = p <0.001; significant difference when compared with the MCL group. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

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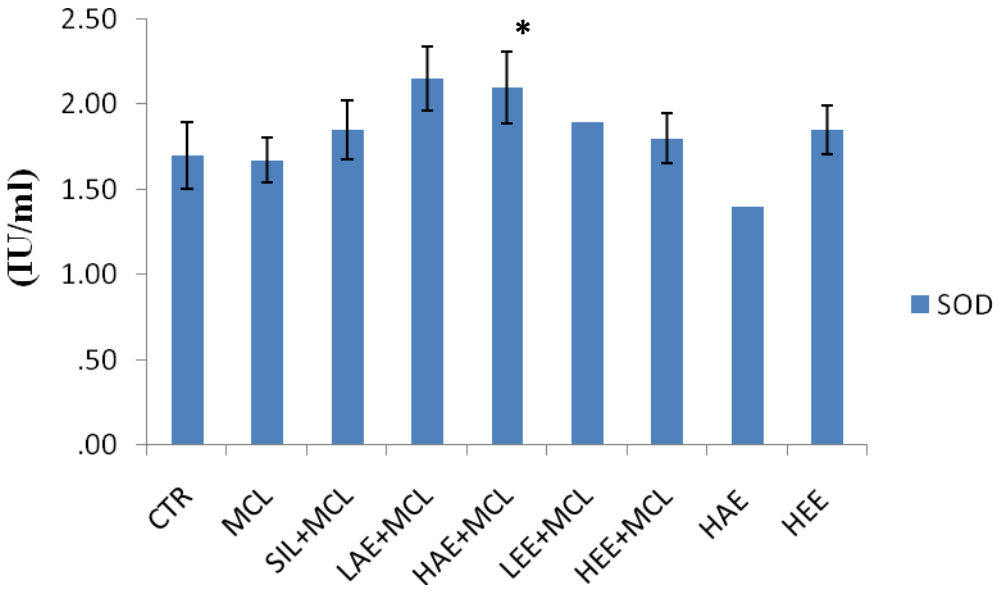


Figure 4.7: **Effect of fruit extracts of *P. dactylifera* on blood serum Superoxide dismutase (SOD) activity of Wistar rats**

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test:* ***** = p <0.05; significant difference when compared with the *control*; ***a*** = p <0.05; significant difference when compared with the MCL group. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

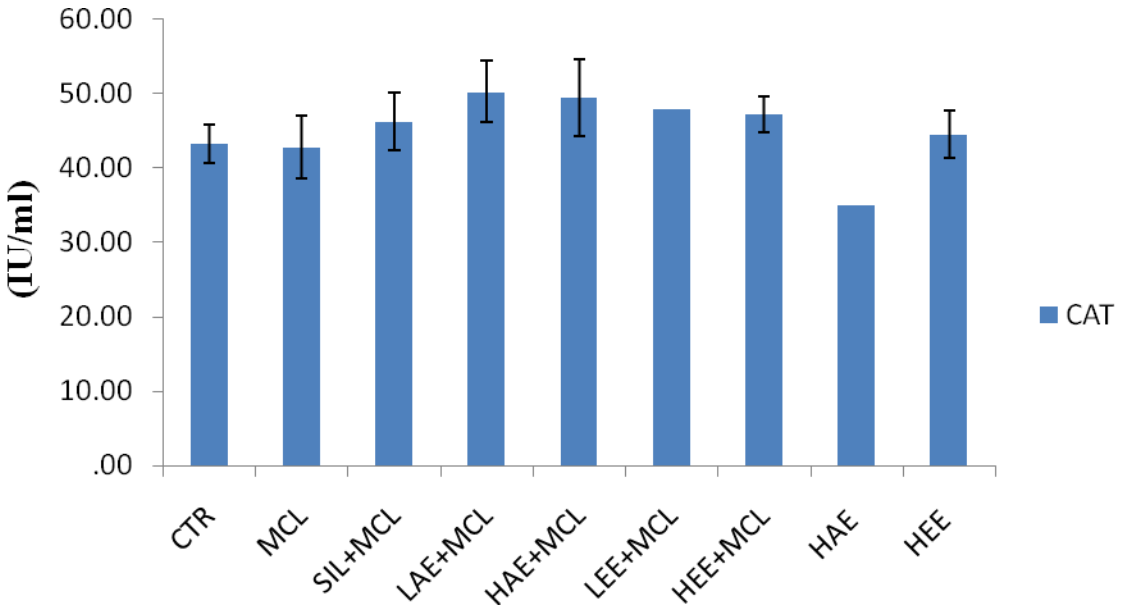


Figure 4.8: Effect of fruit extracts of *P. dactylifera* on blood serum Catalase (CAT) activity of Wistar rats

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test: p* > 0.05; when compared with the *control*. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

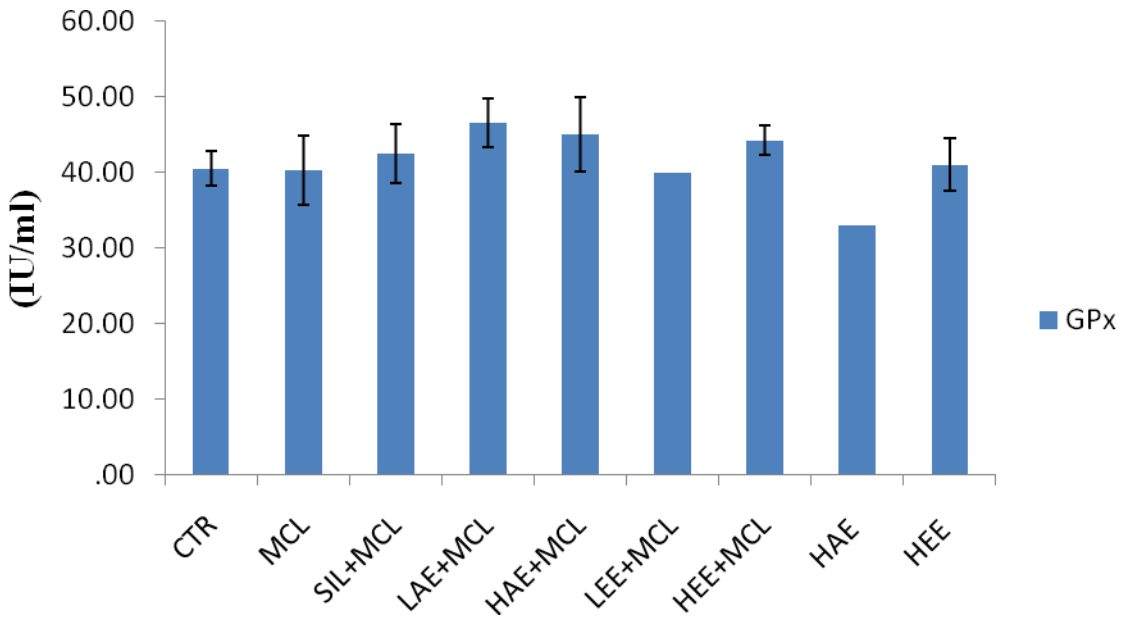


Figure 4.9: **Effect of fruit extracts of *P. dactylifera* on blood serum Glutathione peroxidase (GPx) activity of Wistar rats**

n=5, values are mean ± SEM. One way ANOVA *LSD post hoc test: p* > 0.05; when compared with the *control*. **CTR**= Control (distilled H_2O 0.5 ml/kg); **LAE and HAE=** *Aqueous fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively); **LEE and HEE=** *Ethanol fruit extract of P. dactylifera* (500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kgbw); **SIL**= Silymarin **(**100mg/kg).

4.5 Histopathological Studies of the Liver

Histological sections of Wistar rats' liver stained with routine Haematoxylin and Eosin, (H&E) histological and histochemical Periodic Acid Schiff, (PAS) and Gordon and Sweet, (GS) stains were examined under the light microscope and revealed the following:

The results of the histological observation of the liver sections of rats in the Control Group (Group 1) showed normal histoarchitecture of the liver parenchyma as seen by the characteristic appearance of the hepatic lobule unit, centrilobular venules (central veins), array of anastomosing plates of liver cells (hepatocytes) radiating from the central vein separated by vascular spaces (sinusoids) and portal triad (hepatic portal vein, hepatic artery and bile duct). Histochemical (PAS) stain for demonstrating glycogen revealed PAS positively stained hepatocytes demonstrating the presence of glycogen and Gordon and Sweet stain for reticular fibres revealed normal reticulin framework as shown in Plates 4.1- 4.3.

In the group treated with $HgCl_2$ (Group II), histological observation revealed severe histoarchitectural distortion of the liver parenchyma, degenerative changes such as hepatocellular vacuolation; sinusoidal dilatation, pyknotic nuclei and necrosis as compared the Control. Reduced PAS positive stain intensity was observed indicating glycogen moiety depletion in hepatocytes and GS stain revealed disintegrating reticulin framework when compared to the Control as shown in Plates 4.4 - 4.6.

The results of the liver sections of Wistar rats treated with silymarin, aqueous fruit extracts of *P. dactylifera* (*AFPD)* and ethanol fruit extracts of *P. dactylifera* (EFPD) followed by the administration of $HgCl_2$ showed mild distortion of the histoarchitecture/ cytoarchitecture of the liver. Silymarin-treated followed by $HgCl_2$ (Group III) showed hepatocellular vacuolation when compared to the Control. GS stain showed disoriented reticular fibres (RF) while PAS positive stain intensity was comparable to the

Control as shown in Plates 4.7 - 4.9.

AFPD treated followed by $HgCl_2$ treatment (Groups IV and V), were compareable to the control. GS stain showed disintegrating RF, while PAS positive stain intensity was comparable to the Control as shown in Plates 4.10 - 4.15.

EFPD treated followed by $HgCl_2$ treatment (Groups VI and VII), showed mild degenerative changes, such as sinusoidal dilatation and congested vein. GS stained section was comparable to the Control while reduced PAS positive stain intensity showed depletion of glycogen in hepatocytes as compared to the Control as shown in Plates 4.16 - 4.21.

Histological and histochemical (PAS and GS) examinations of the liver sections of Wistar rats treated with AFPD (Group VIII) and EFPD (Group IX) only revealed mild distortion of the histoarchitecture/ cytoarchitecture of the liver such as hepatocellular vacuolation, depletion of glycogen in hepatocytes due to reduced PAS positive stain intensity and distortion of RF when compared to the Control as shown in Plates 4.22 - 4.27.

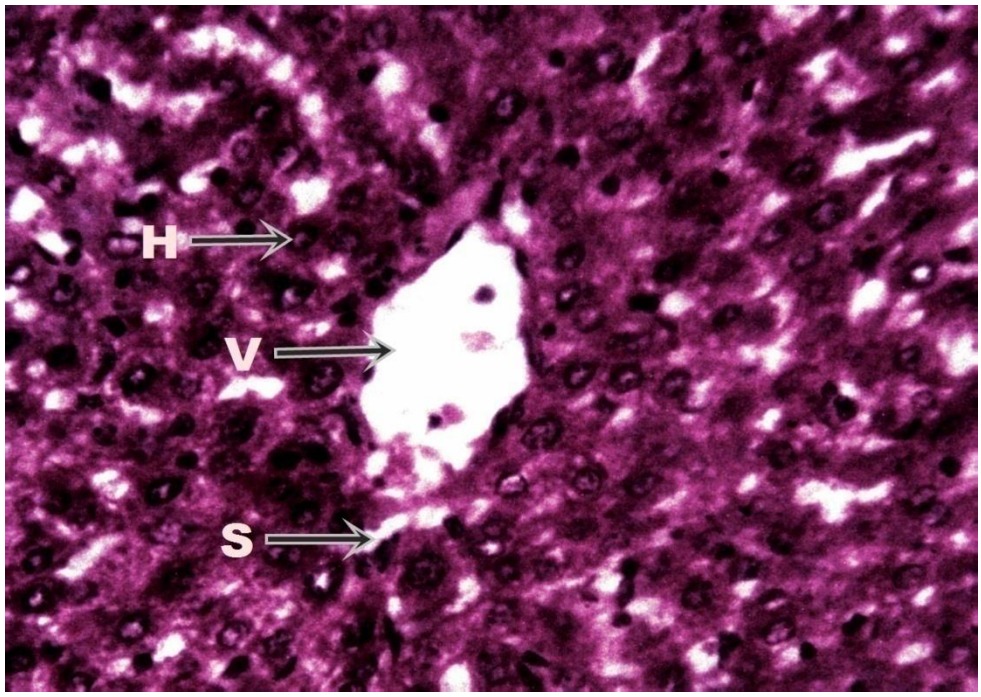


Plate 4.1: Liver transverse section of Wistar rat of Control Group; showing normal liver histoarchitecture. Central vein (V); Hepatocyte (H); Sinusoid (S). H and E; (Mag x 400).

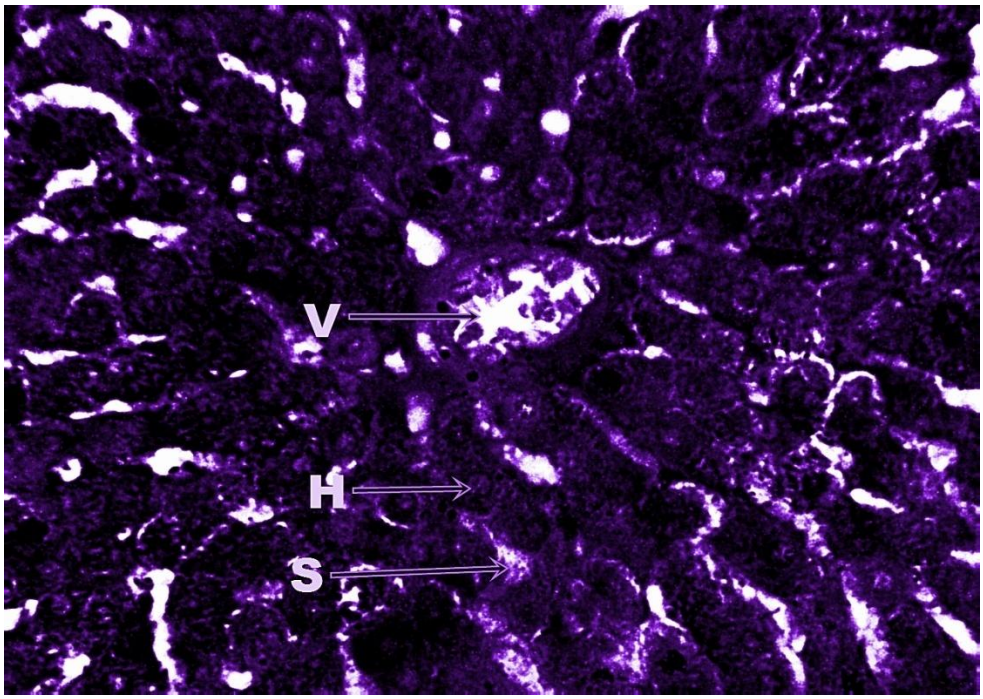


Plate 4.2: Liver transverse section of Wistar rat of Control Group showing; positive PAS stain intensity signifying the presence of glycogen. Central vein (V); Hepatocyte (H); Sinusoid (S). PAS; (Mag x 400).

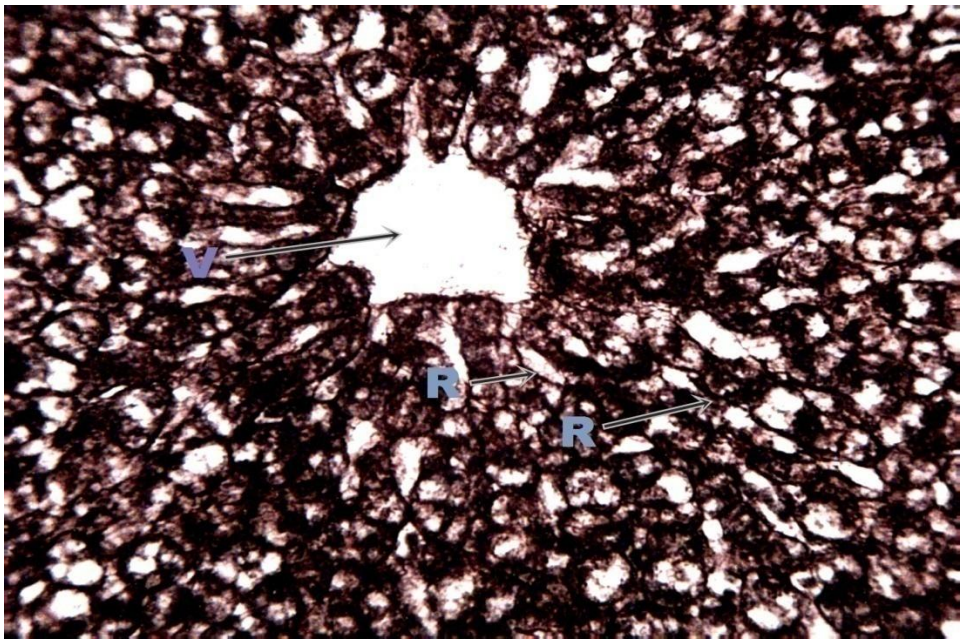


Plate 4.3: Liver transverse section of Wistar rat of Control Group showing; normal reticular framework (R); Central vein (V). Gordon and Sweet; (Mag x 250).

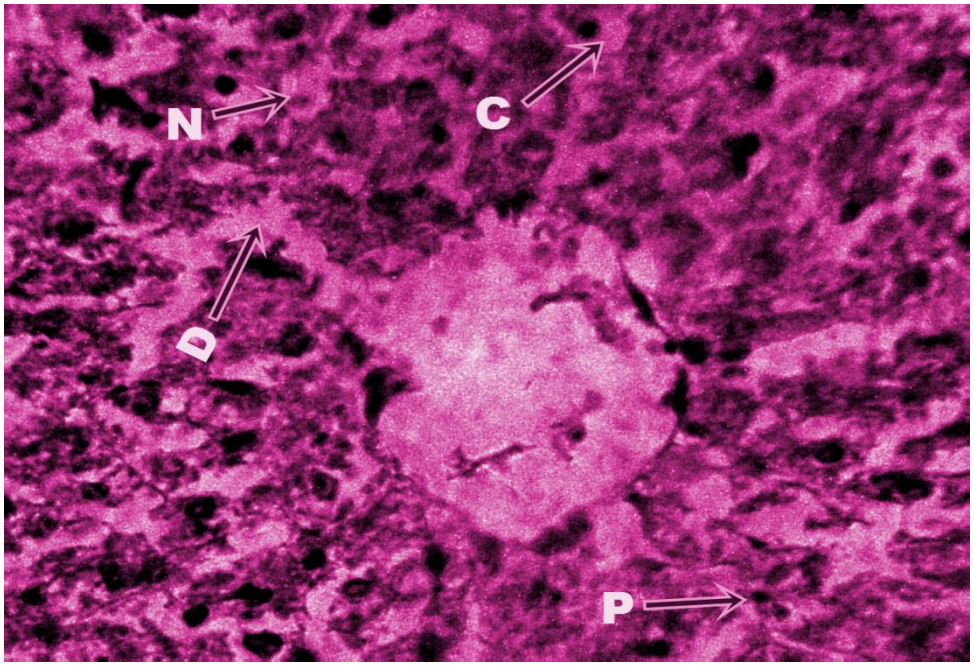


Plate 4.4: Liver transverse section of Wistar rat of Group II demonstrating cytoplasmic vacuolation (C), sinusoidal dilatation (D), pyknotic nucleus (P) and necrosis (N). H and E; (Mag x 400).

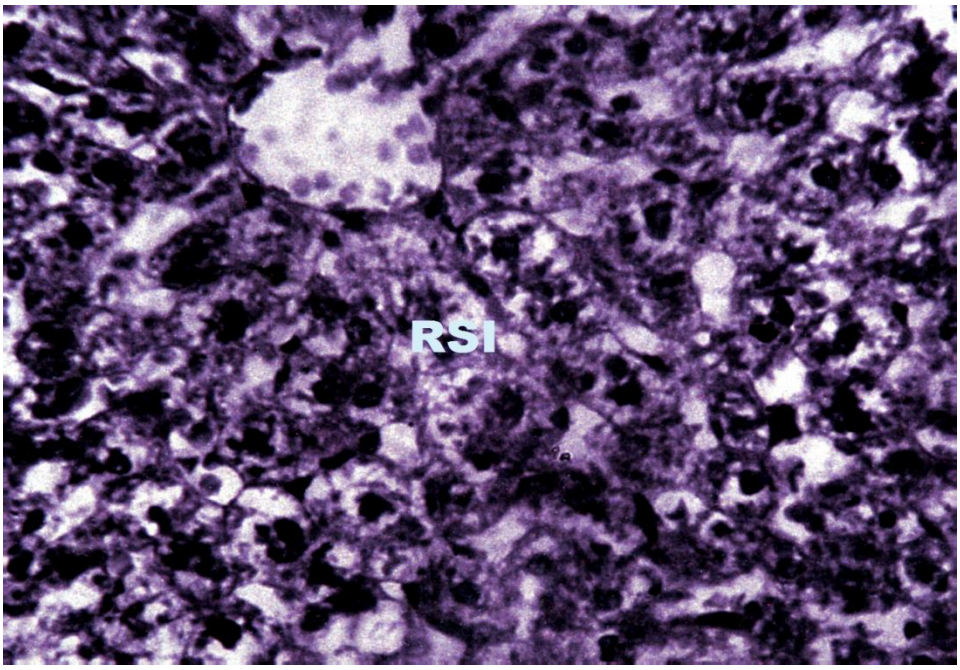


Plate 4.5: Liver transverse section of Wistar rat of Group II demonstrating reduced PAS positive stain intensity (RSI) due to glycogen depletion. PAS; (Mag x 400).

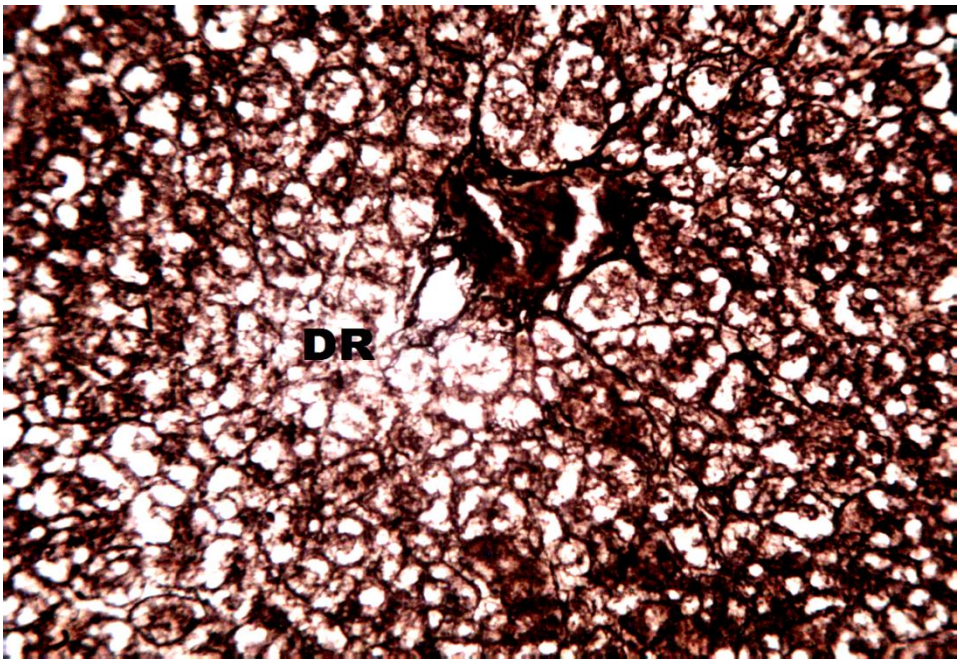


Plate 4.6: Liver transverse section of Wistar rat of Group II demonstrating area of disoriented reticulin framework (DR). Gordon and Sweet; (Mag x 250).

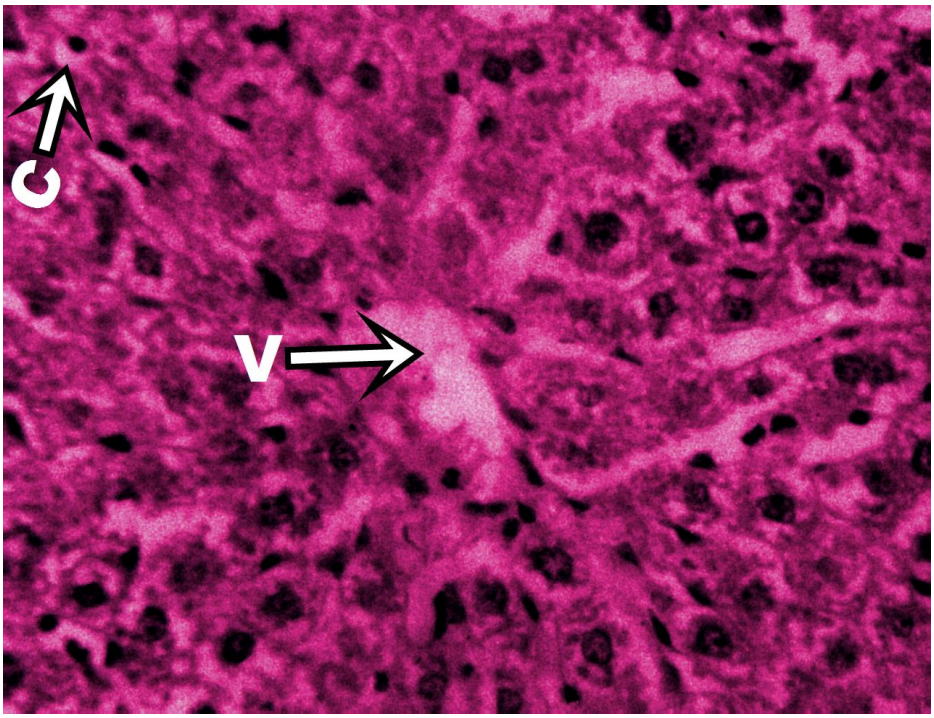


Plate 4.7: Liver transverse section of Wistar rat of Group III demonstrating cytoplasmic vacuolation (C); Central vein (V). H and E; (Mag x400).

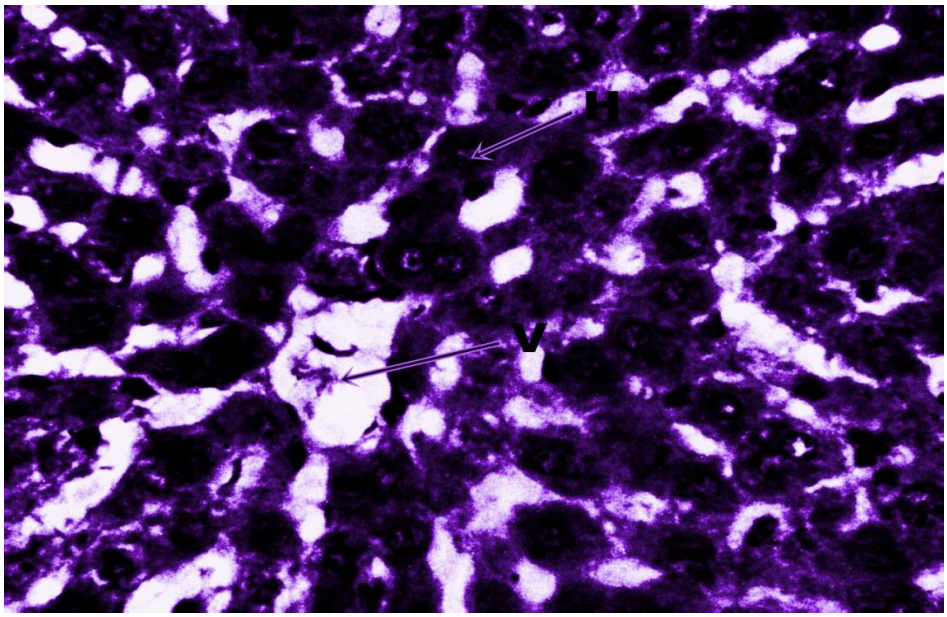


Plate 4.8: Liver transverse section of Wistar rat of Group III demonstrating PAS positive stain intensity. Central vein (V); Hepatocyte (H). PAS; (Mag x400).

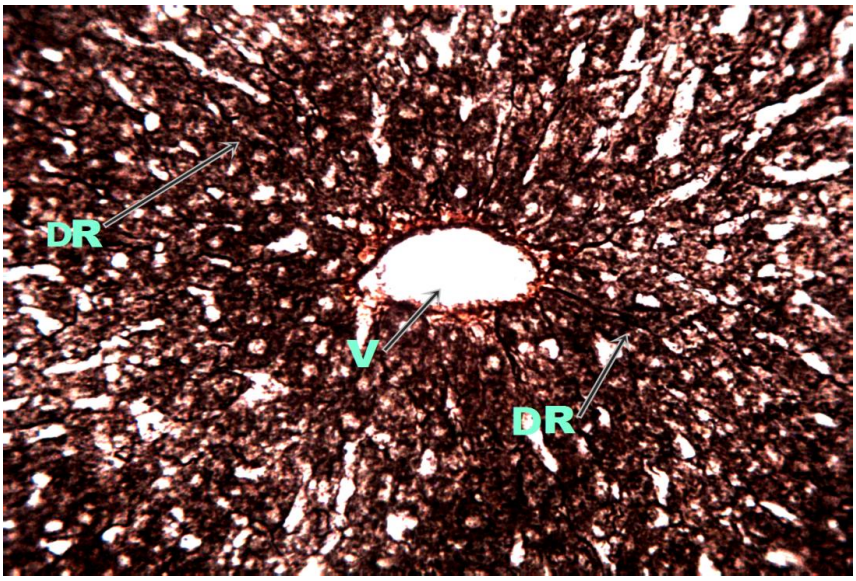


Plate 4.9: Liver transverse section of Wistar rat of Group III demonstrating disoriented reticulin framework (DR). Central vein (V). Gordon and Sweet; (Mag x250).

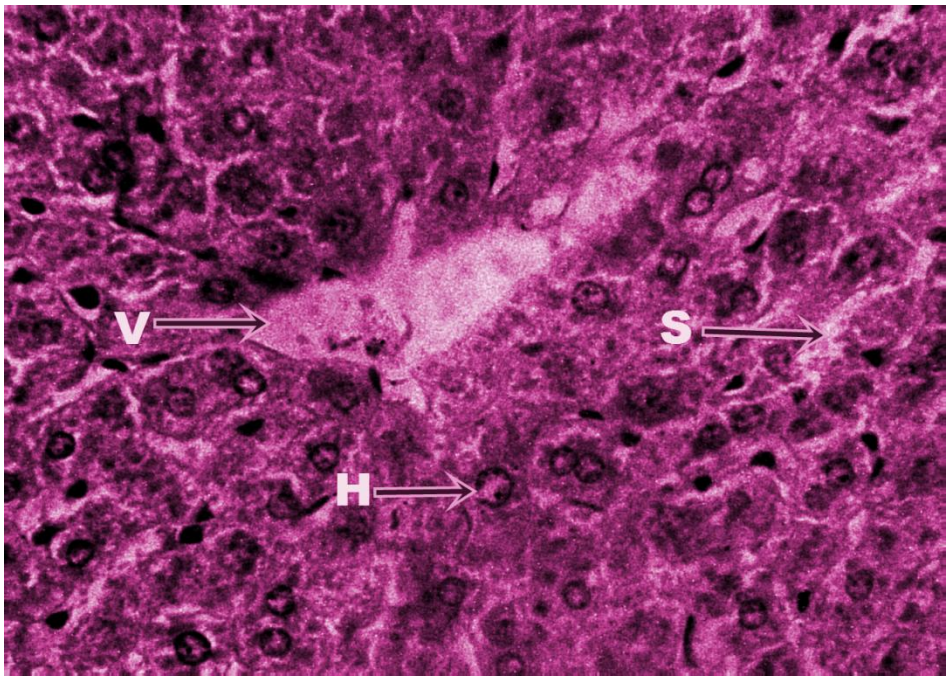


Plate 4.10: Liver transverse section of Wistar rat of Group IV demonstrating normal liver histoarchitecture. Central vein (V); Hepatocyte (H); and Sinusoid (S). H and E; (Mag x 400).

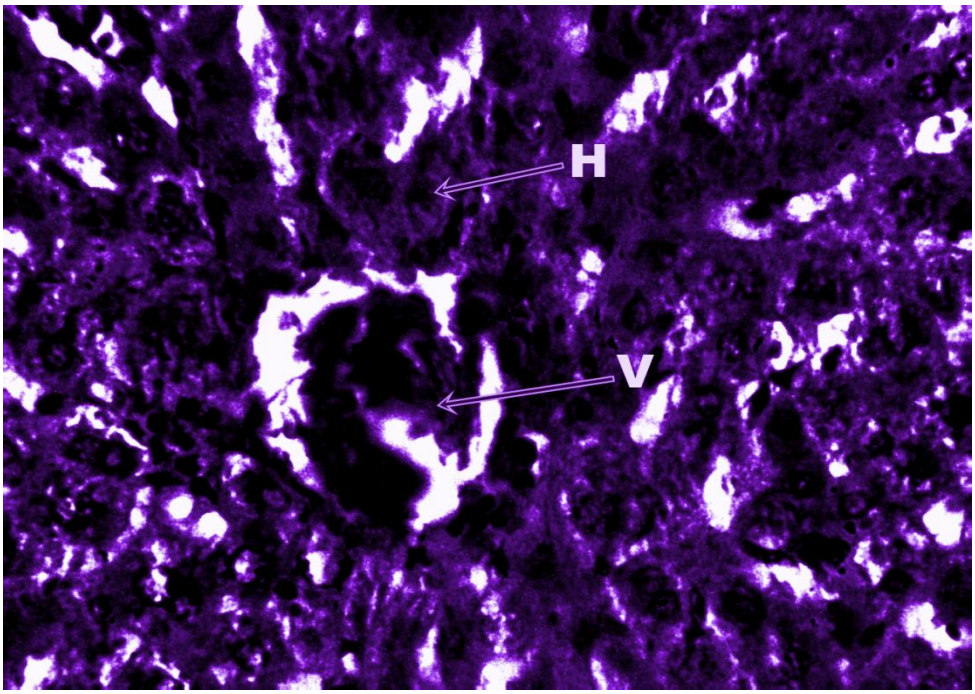


Plate 4.11: Liver transverse section of Wistar rat of Group IV demonstrating PAS positive stain intensity. Central vein (V); Hepatocyte (H); and Sinusoid (S). PAS; (Mag x 400).

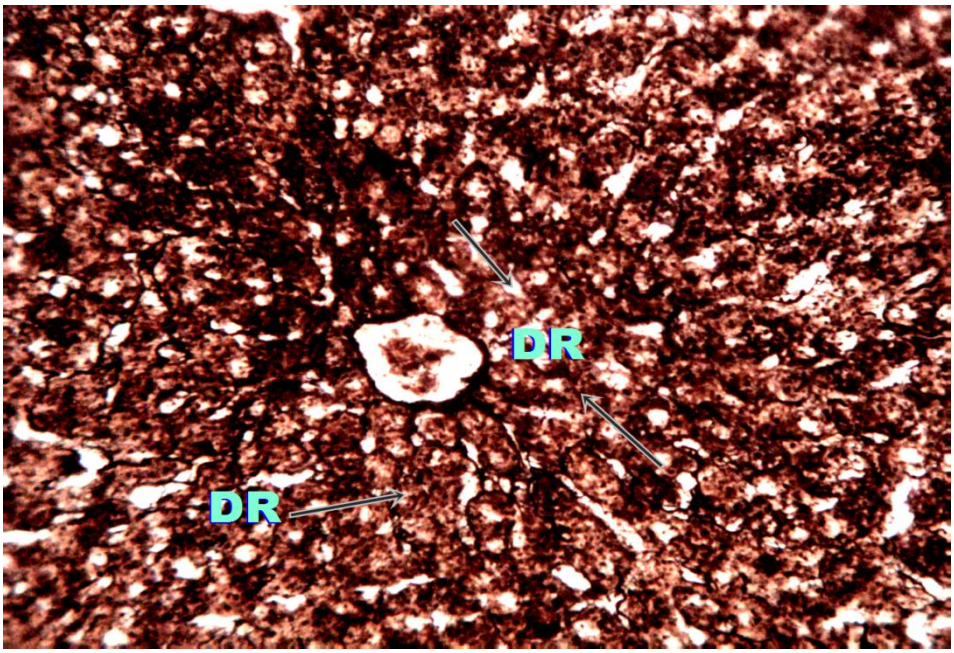


Plate 4.12: Liver transverse section of Wistar rat of Group IV demonstrating disintegrated reticulin fiber (DR). Gordon and Sweet; (Mag x 250).

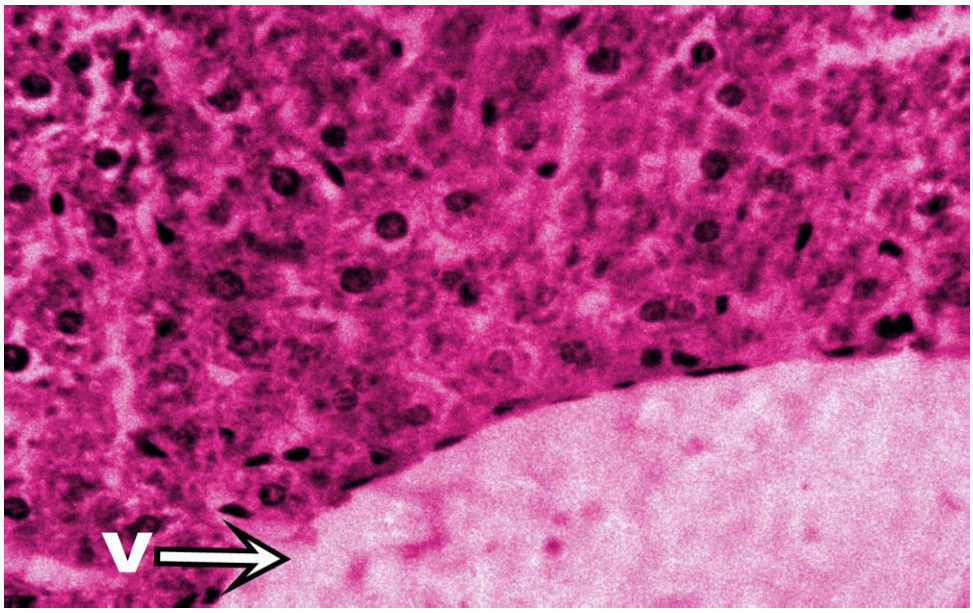


Plate 4.13: Liver transverse section of Wistar rat of Group V demonstrating Central vein (V). H and E; (Mag x 400).

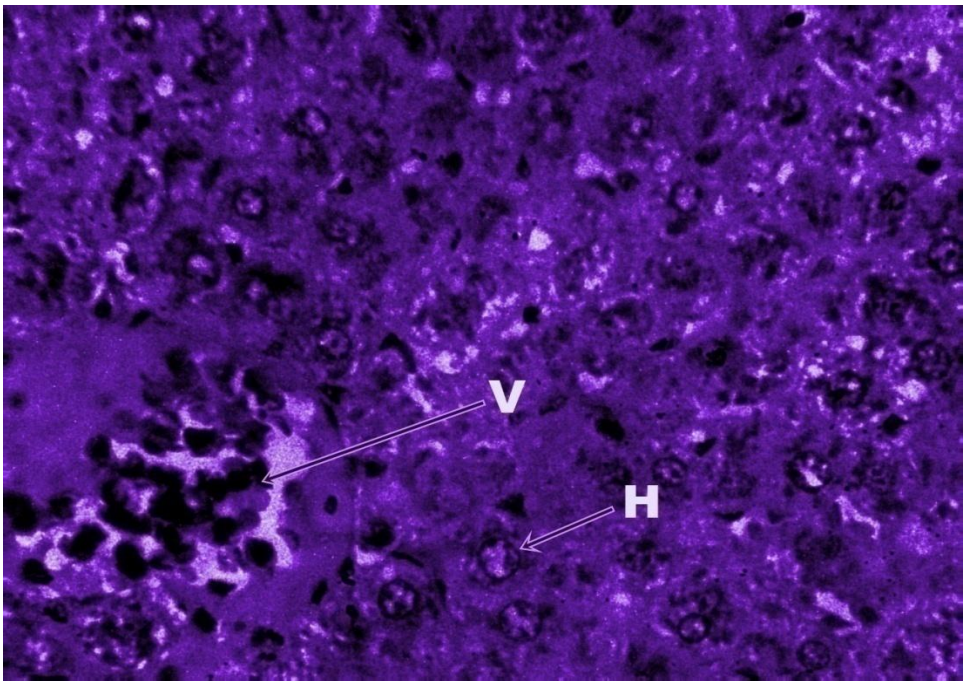


Plate 4.14: Liver transverse section of Wistar rat of Group V demonstrating PAS positive stain intensity. Central vein (V); Hepatocyte (H). PAS; (Mag x 400).

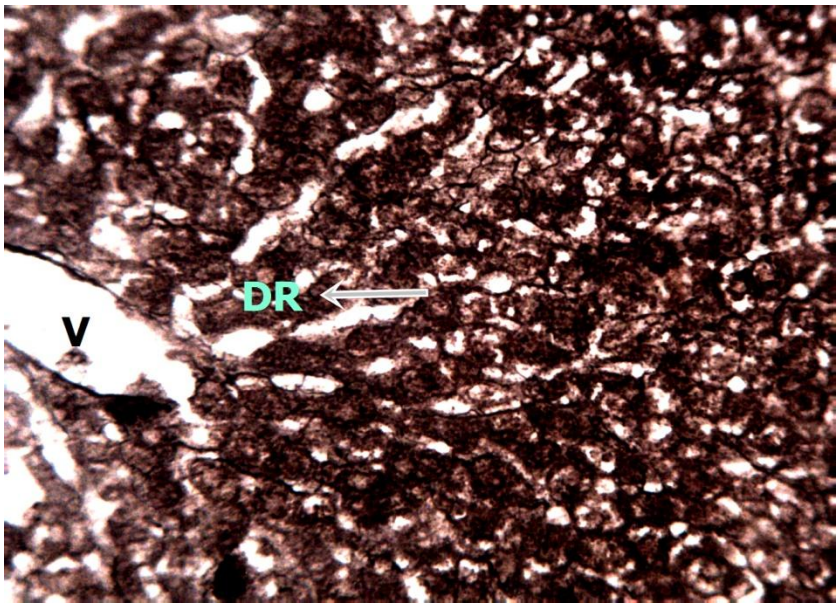


Plate 4.15: Liver transverse section of Wistar rat of Group V demonstrating disintegrated reticulin fibers (DR); Central vein (V). Gordon and Sweet; (Mag x 250).

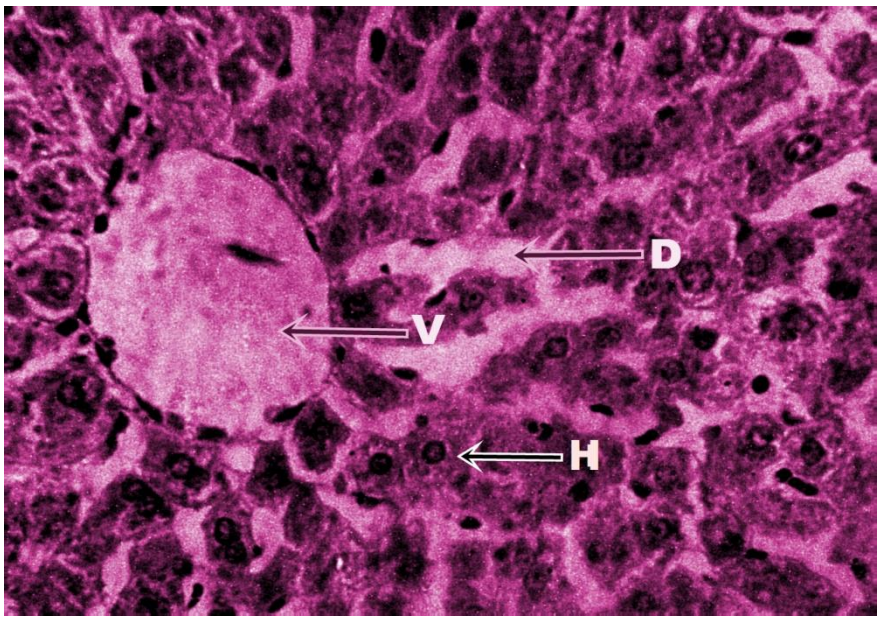


Plate 4.16: Liver transverse section of Wistar rat of Group VI demonstrating sinusoidal dilatation (D); Central vein (V); Hepatocyte (H). H and E; (Mag x 400).

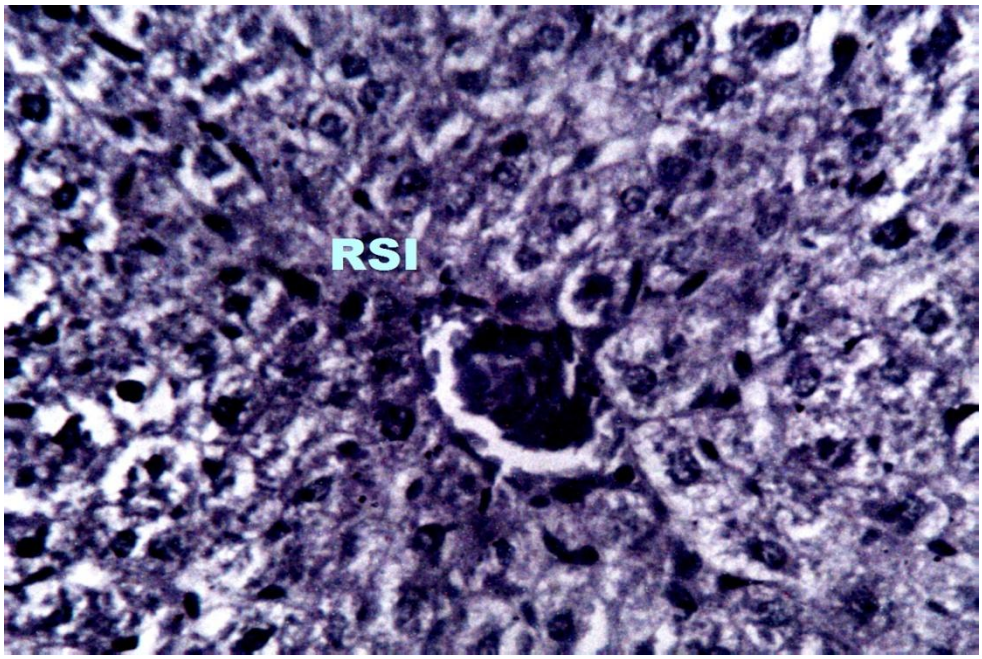


Plate 4.17: Liver transverse section of Wistar rat of Group VI demonstrating reduced PAS positive stain intensity (RSI) due to glycogen depletion. PAS; (Mag x 400).

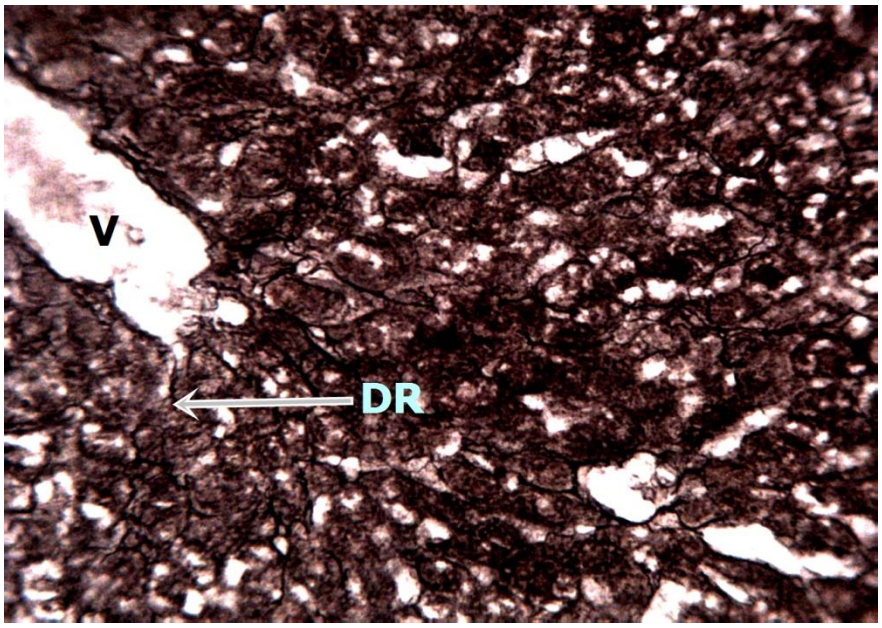


Plate 4.18: Liver transverse section of Wistar rat of Group VI demonstrating disoriented reticulin framework (DR); Central vein (V). Gordon and Sweet; (Mag x 250)

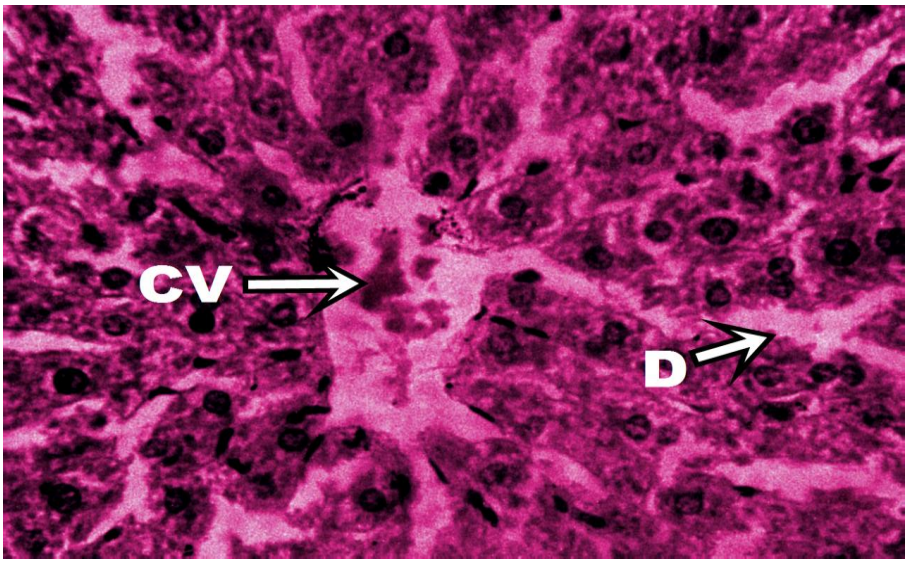


Plate 4.19: Liver transverse section of Wistar rat of Group VII demonstrating congested vein (CV); sinusoidal dilatation (D). H and E; (Mag x 400).

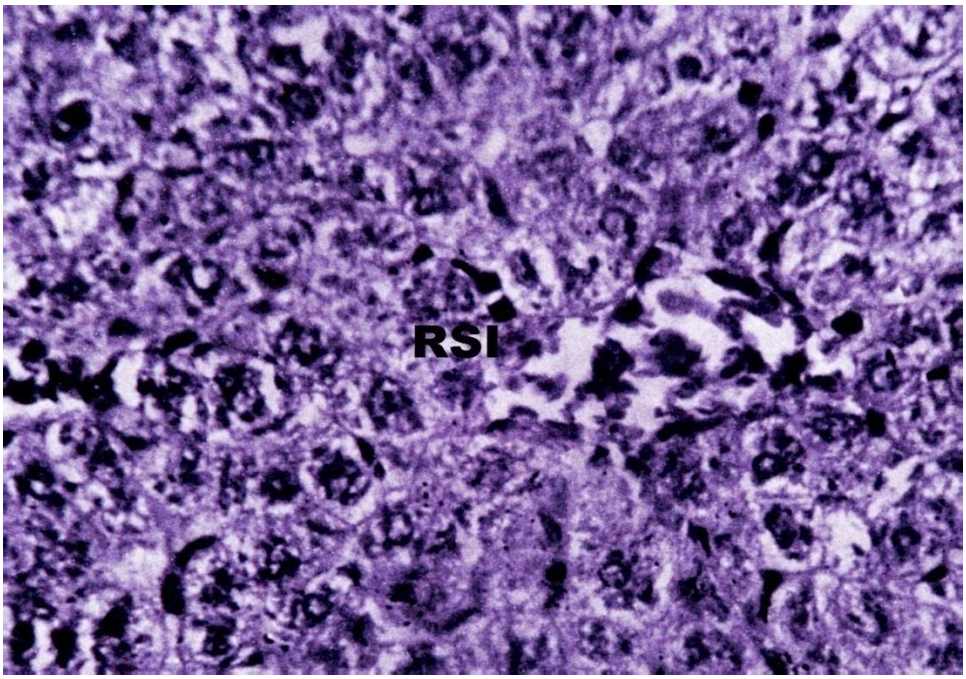


Plate 4.20: Liver transverse section of Wistar rat of Group VII demonstrating reduced PAS positive stain intensity (RSI). PAS; (Mag x 400).

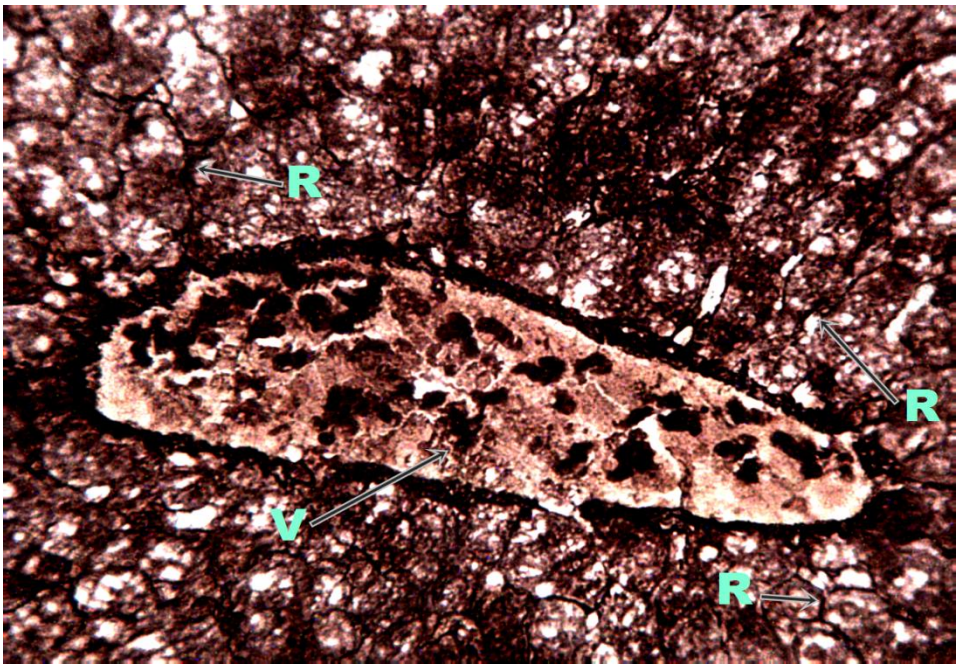


Plate 4.21: Liver transverse section of Wistar rat of Group VII demonstrating normal reticulin framework (R). Central vein (V). Gordon and Sweet; (Mag x250).

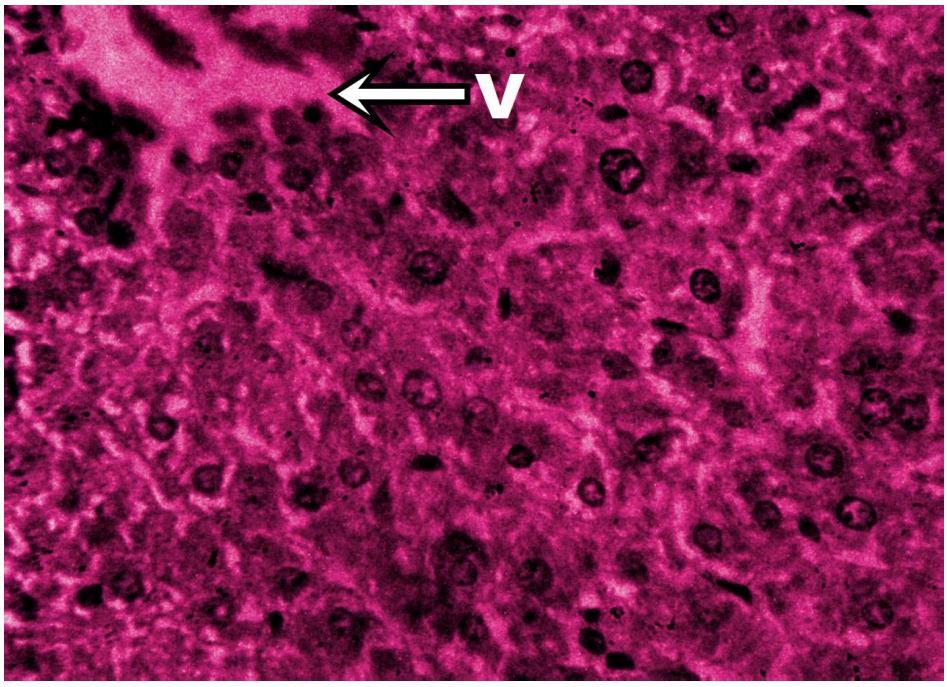


Plate 4.22: Liver transverse section of Wistar rat of Group VIII demonstrating Central vein (V); H and E; (Mag x400).

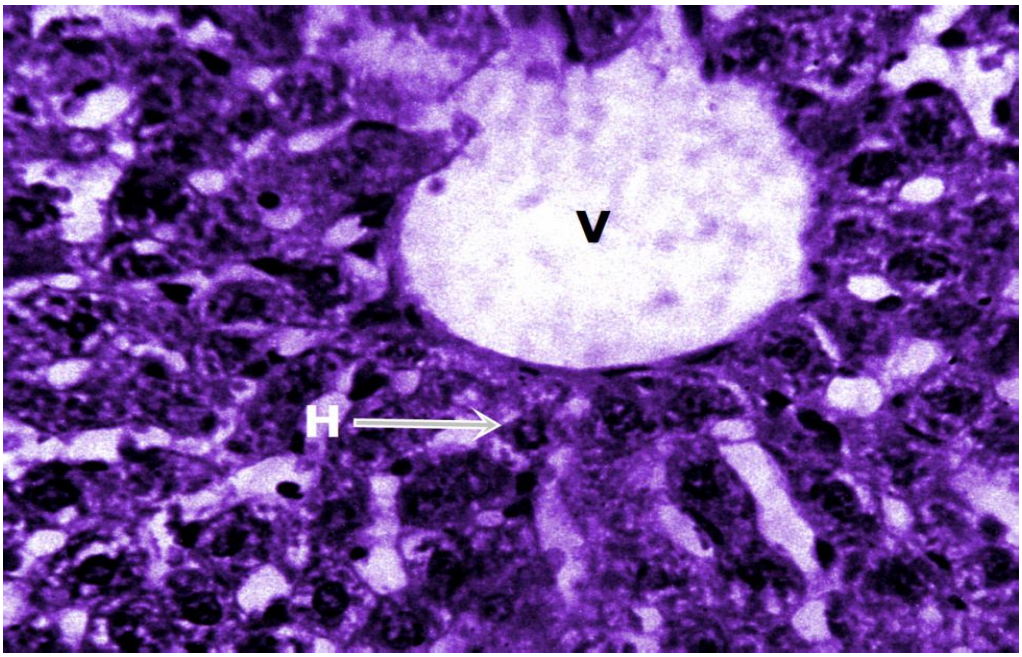


Plate 4.23: Liver transverse section of Wistar rat of Group VIII demonstrating PAS positive stain intensity. Central vein (V); Hepatocyte (H). PAS; (Mag x400).

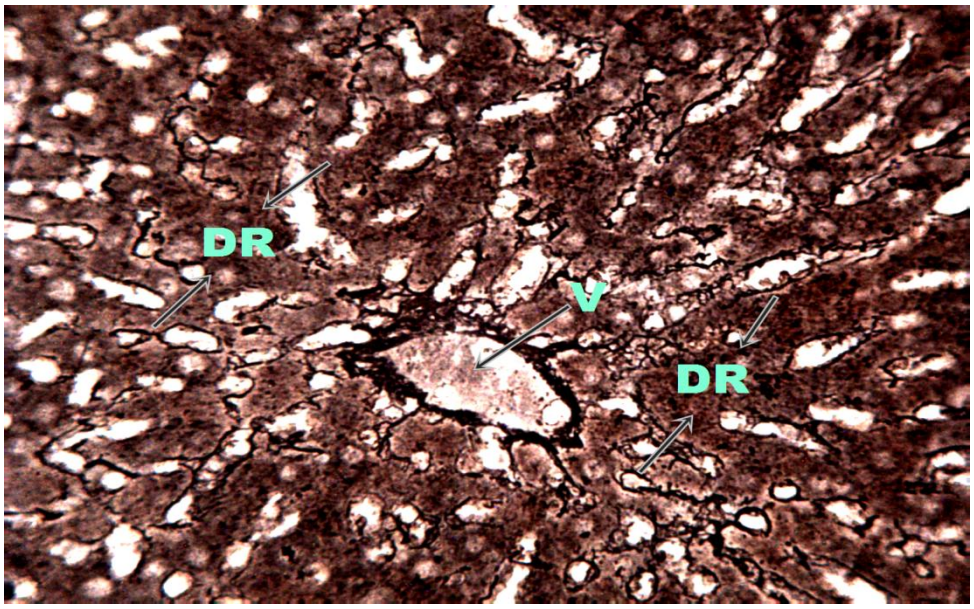


Plate 4.24: Liver transverse section of Wistar rat of Group VIII demonstrating disintegrated reticulin fibre (DR). Central vein (V). Gordon and Sweet; (Mag x250).

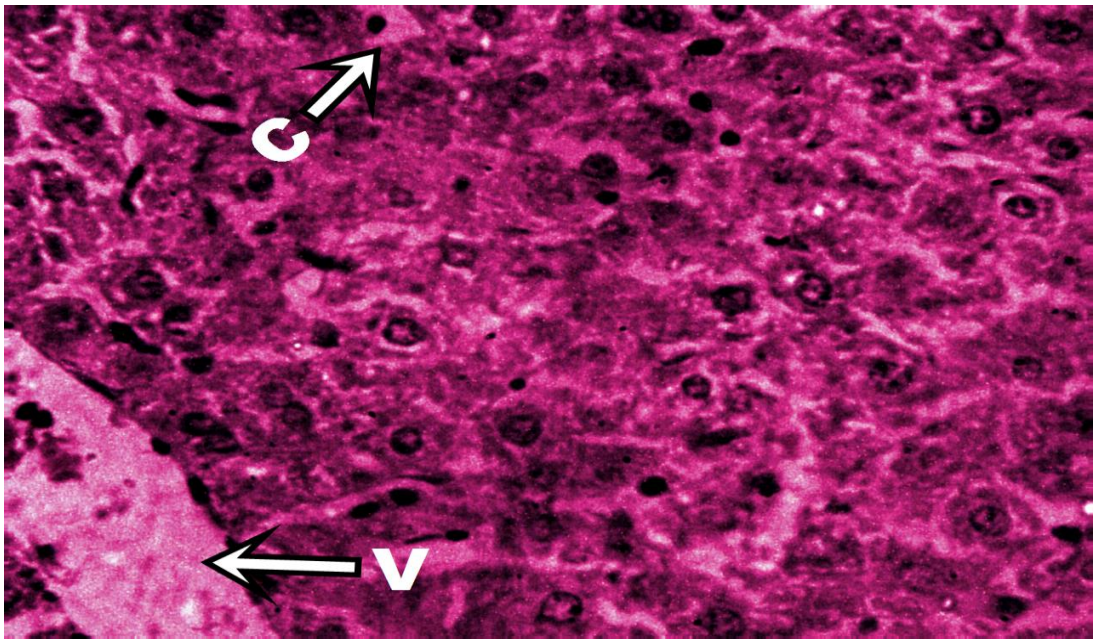


Plate 4.25: Liver transverse section of Wistar rat of Group IX demonstrating cytoplasmic vacuolation (C). Central vein (V). H and E; (Mag x400).

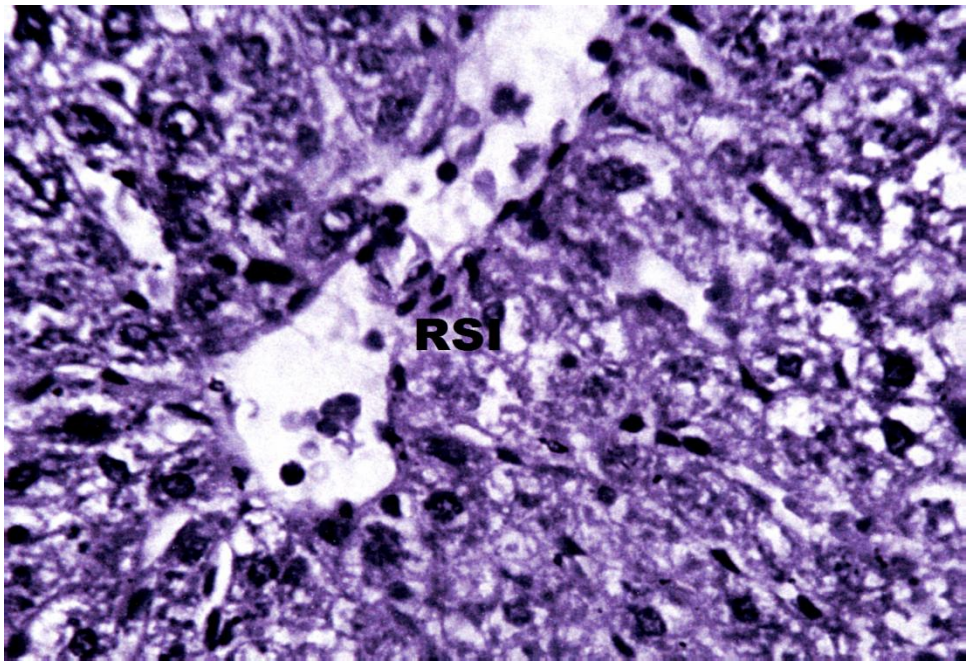


Plate 4.26: Liver transverse section of Wistar rat of Group IX demonstrating reduced PAS stain intensity (RSI) due to depletion of glycogen. PAS; (Mag x400).

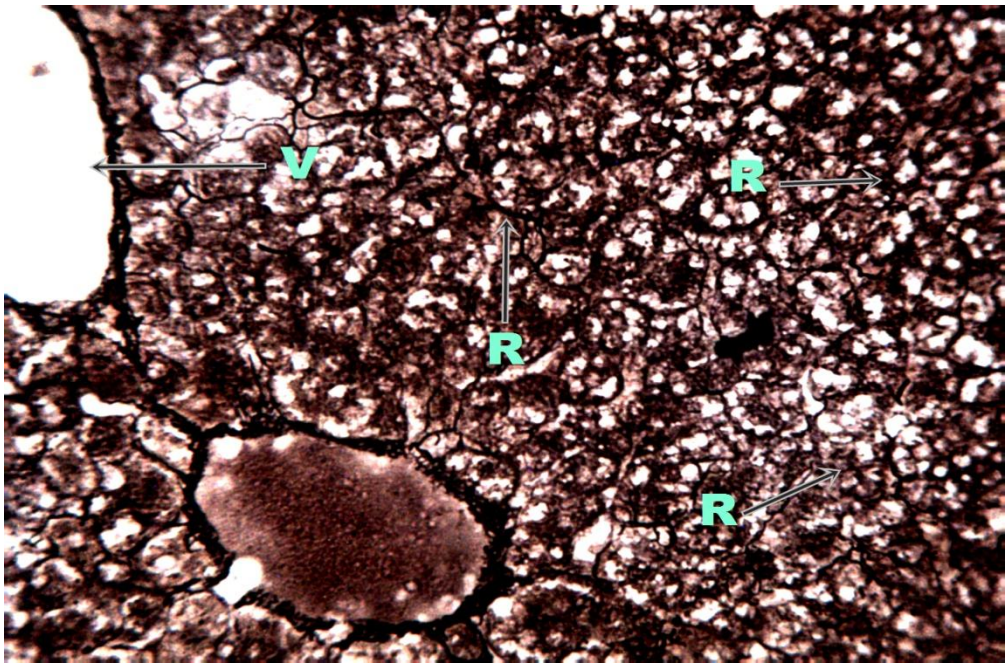


Plate 4.27: Liver transverse section of Wistar rat of Group IX demonstrating normal reticulin framework (R); Central vein (V). Gordon and Sweet; (Mag x250).

4.6 Histological and Histochemical Studies of the Kidney

Histological sections of Wistar rats' kidney stained with routine (Haematoxylin and Eosin, H&E) histological and histochemical (Periodic Acid Schiff, PAS) stain were examined under the light microscope and revealed the following:

The results of the histological observations of the kidney sections of rats in the Control Group (Group 1) showed normal histoarchitecture of the kidney tissue as seen by the characteristic appearance of the renal cortex comprised of the renal corpuscle (Bowman's capsule, Urinary space, glomerulus (anastomosing capillaries), blood vessels, proximal convoluted tubule and distal convoluted tubules and the renal medulla comprised of the thin descending limb, the thin and thick ascending limbs all of the loop of Henle, the collecting tubules, collecting ducts, duct of Bellini and the proximal end of the ureter. PAS staining for glycogen revealed positive staining intensity demonstrating the presence of glycogen as shown in Plates 4.28 - 4.29.

The histological results from the kidney sections treated with $HgCl_2$ (Group 2) revealed severe histoarchitectural and cytoarchitectural distortion of the kidney tissue, degenerative changes such as dilatation of Urinary space, distortion of renal corpuscle (RC), renal tubular necrosis and pyknotic nuclei. Reduced PAS stain intensity was observed as shown in Plates 4.30 - 4.31.

Histological and histochemical (PAS) examinations of the kidney sections of Wistar rats treated with Silymarin, aqueous fruit extracts of *P. dactylifera* (AFPD) and ethanol fruit extracts of *P. dactylifera* (EFPD) followed by the administration of $HgCl_2$ revealed mild distortion of the histoarchitecture/ cytoarchitecture of the kidney. The histological features of the Silymarin treated (Group III) followed by the administration of $HgCl_2$ showed cytoplasmic vacuolation as compared to the Control. PAS positive stain intensity was comparable to the Control as shown in Plates 4.32- 4.33.

AFPD treated (Groups IV and V) followed by the administration of $HgCl_2$ showed mild degenerative changes such as, dilatation of Urinary space. PAS positive stain intensity was comparable to the Control as shown in Plates 4.34 - 4.38.

EFPD treated (Groups VI and VII) followed by the administration of $HgCl_2$ showed degenerative changes, such as dilatation of Urinary space, distortion of RC, renal tubular necrosis and cytoplasmic vacuolation, pyknotic nuclei. Reduced PAS staining intensity revealed glycogen depletion as shown in Plates 4.39-4.42.

Histological and histochemical (PAS) examinations of the results of the kidney sections of Wistar rats treated with AFPD (Group VIII) and EFPD (Group IX) only revealed mild distortion of the histoarchitecture of the kidney which manifested as dilatad Urinary space. PAS positive stain intensity was comparable to the Control as shown in Plates 4.43 - 4.46.

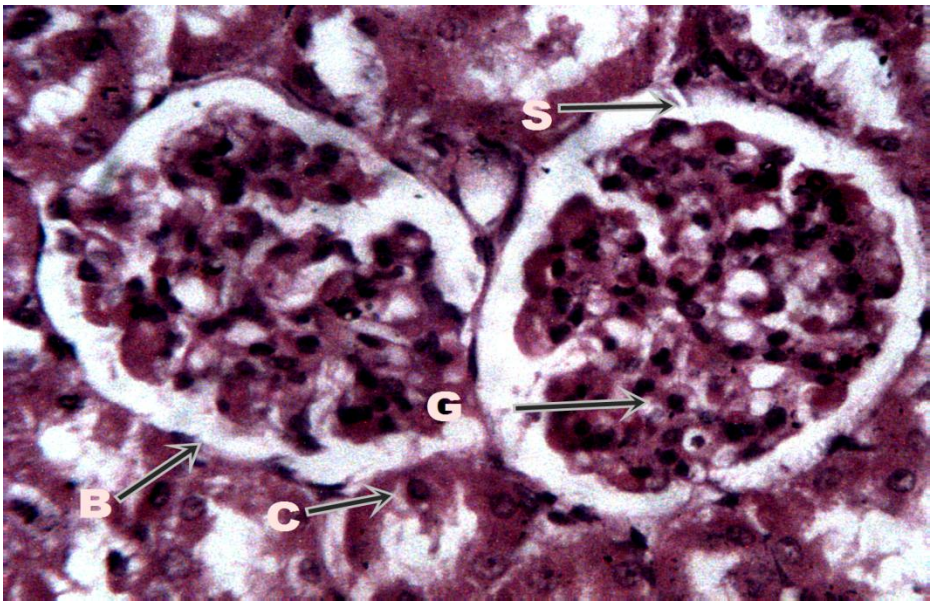


Plate 4.28: Kidney transverse section of Wistar rat of Control Group demonstrating normal histology. Glomerulus (G); Bowman's capsule (B); Urinary space (S) and renal tubular cell (C). H and E; (Mag x400).

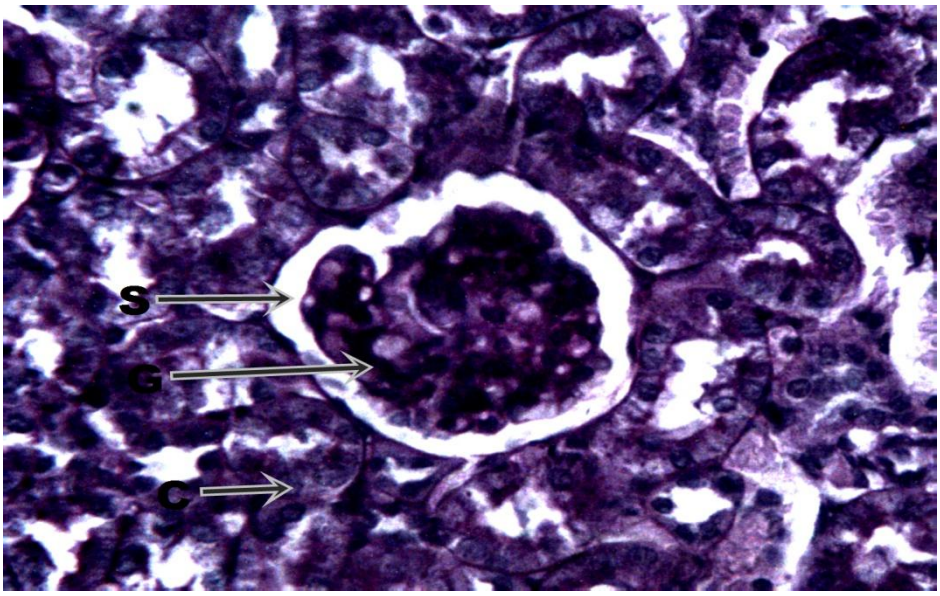


Plate 4.29: Kidney transverse section of Wistar rat of Control Group demonstrating PAS positive stain intensity. Glomerulus (G); Urinary space (S) and renal tubular cell (C). PAS; (Mag x400).

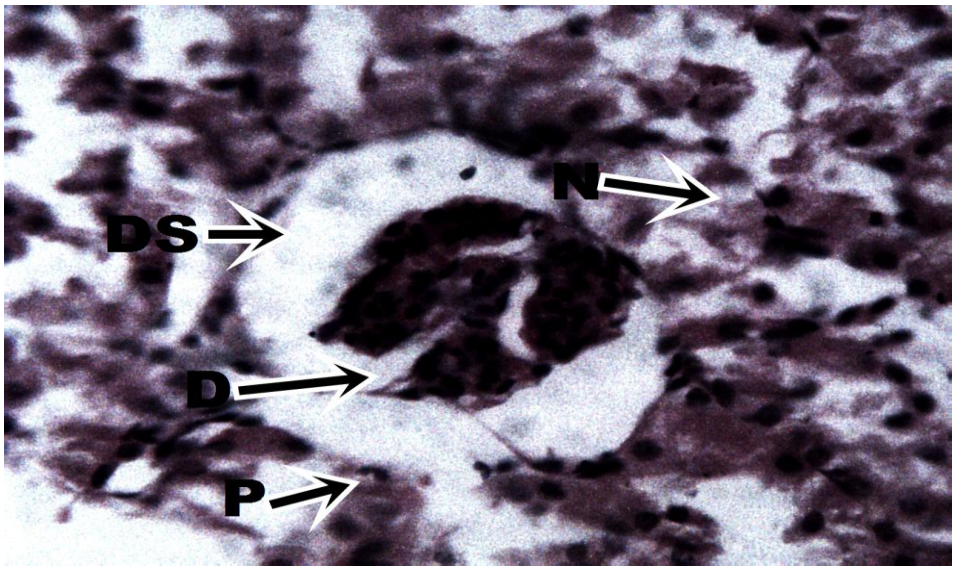


Plate 4.30: Kidney transverse section of Wistar rat of Group II demonstrating histoarchitectural distortion. Dilated Urinary space (DS); Distorted glomerulus (D); Pyknotic nucleus (P) and Necrosis (N). H and E; (Mag x400).

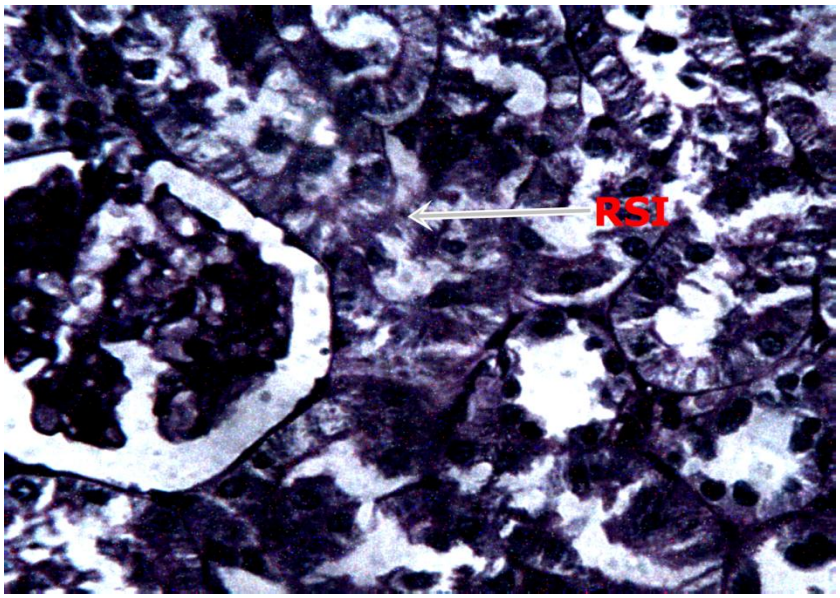


Plate 4.31: Kidney transverse section of Wistar rat treated with $HgCl_2$ (5 mg/kgbw) demonstrating reduced PAS positive stain intensity (RSI) due to depletion of glycogen. PAS; (Mag x400).

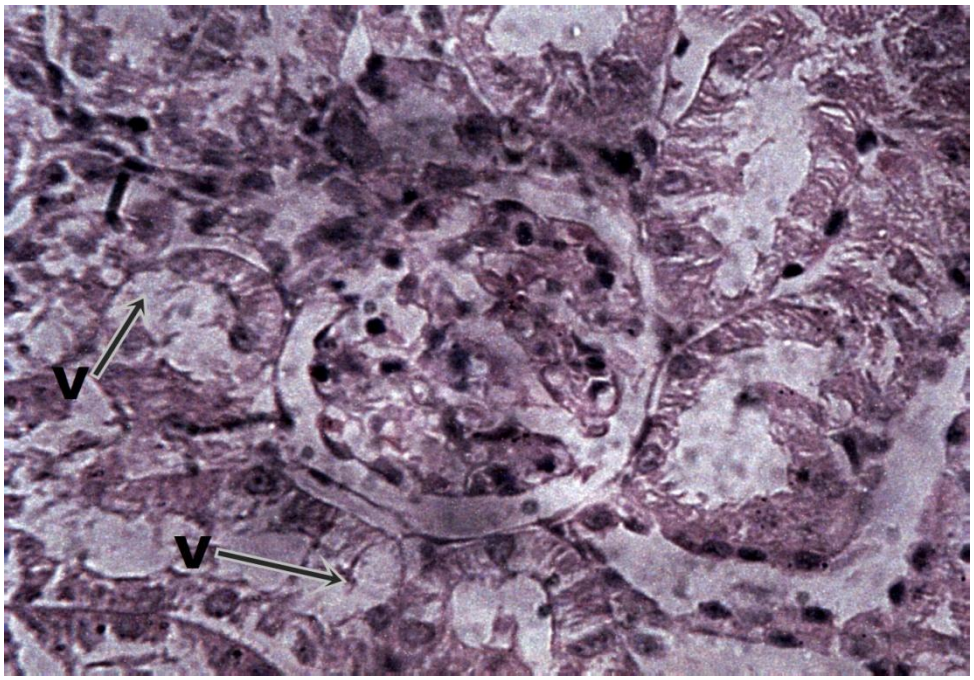


Plate 4.32: Kidney transverse section of Wistar rat of Group III demonstrating cytoplasmic vacuolation (V). H and E; (Mag x400).

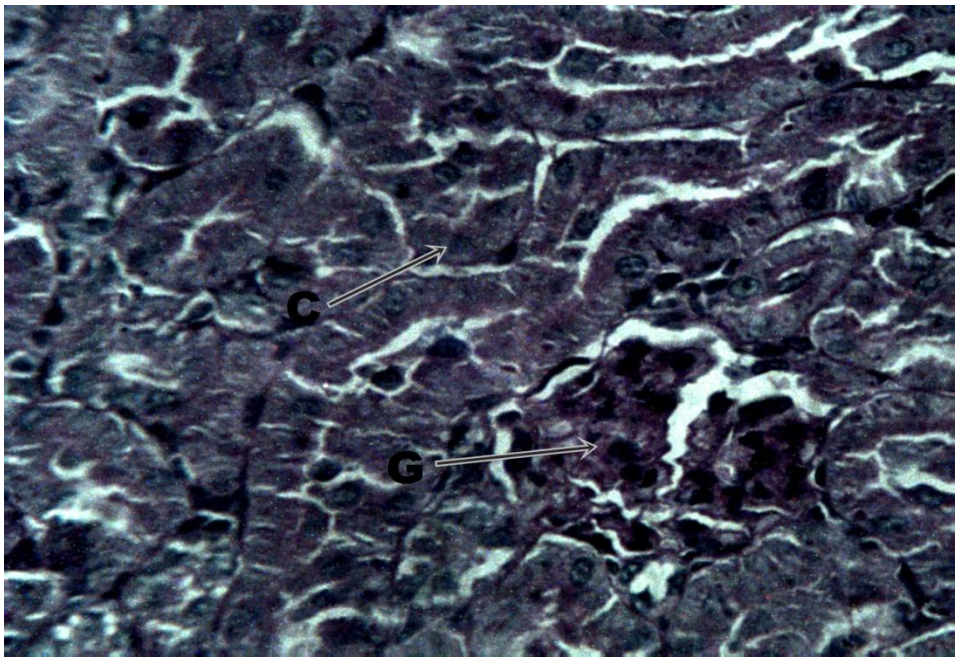


Plate 4.33: Kidney transverse section of Wistar rat of Group III demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS; (Mag x400).

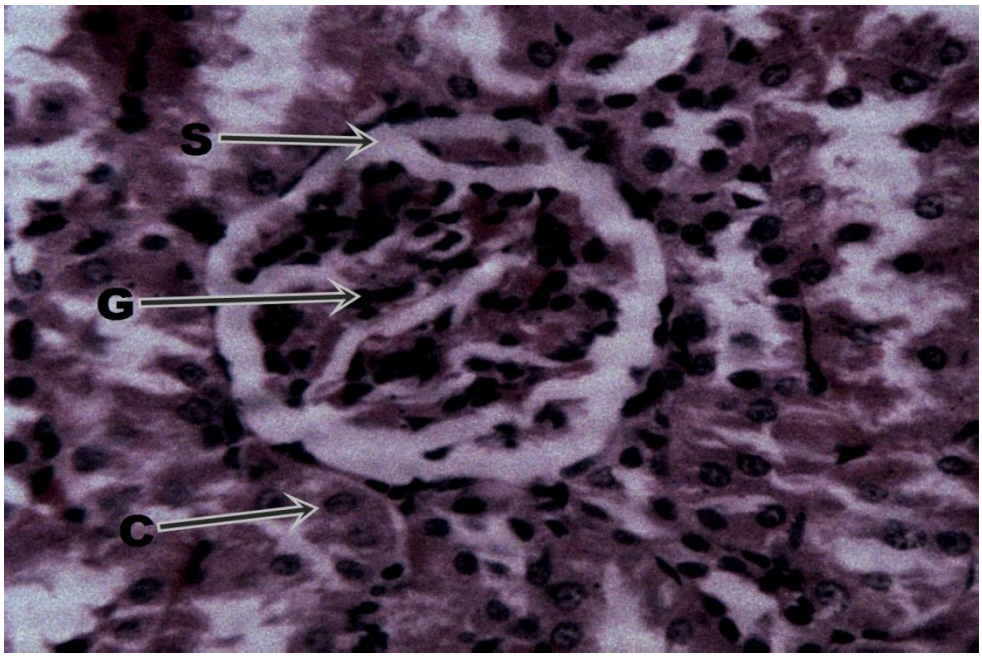


Plate 4.34: Kidney transverse section of Wistar rat of Group IV demonstrating normal histoarchitecture. Renal tubular cell (C); Glomerulus (G); Urinary space (S). H and E; (Mag x400).

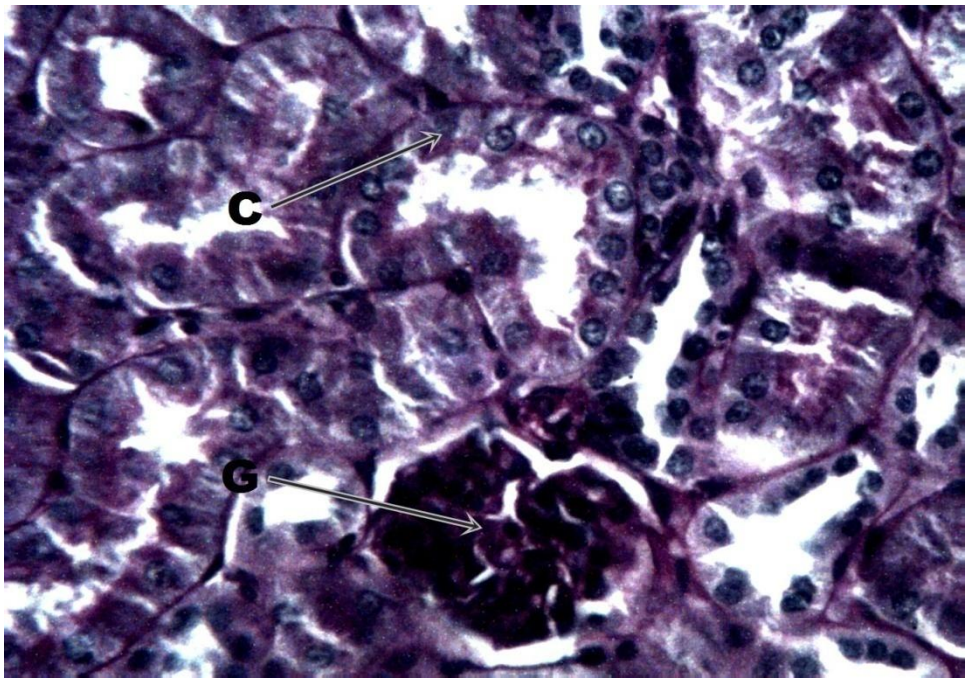


Plate 4.35: Kidney transverse section of Wistar rat of Group IV demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS; (Mag x400).

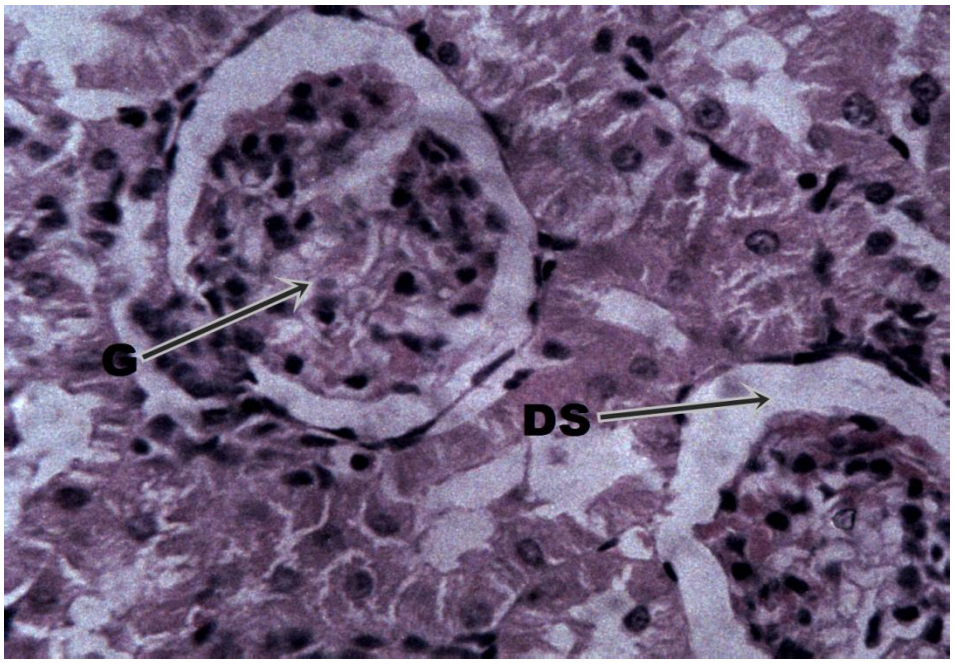


Plate 4.36: Kidney transverse section of Wistar rat of Group V demonstrating dilated Urinary space (DS); Glomerulus (G). H and E, (Mag x400).

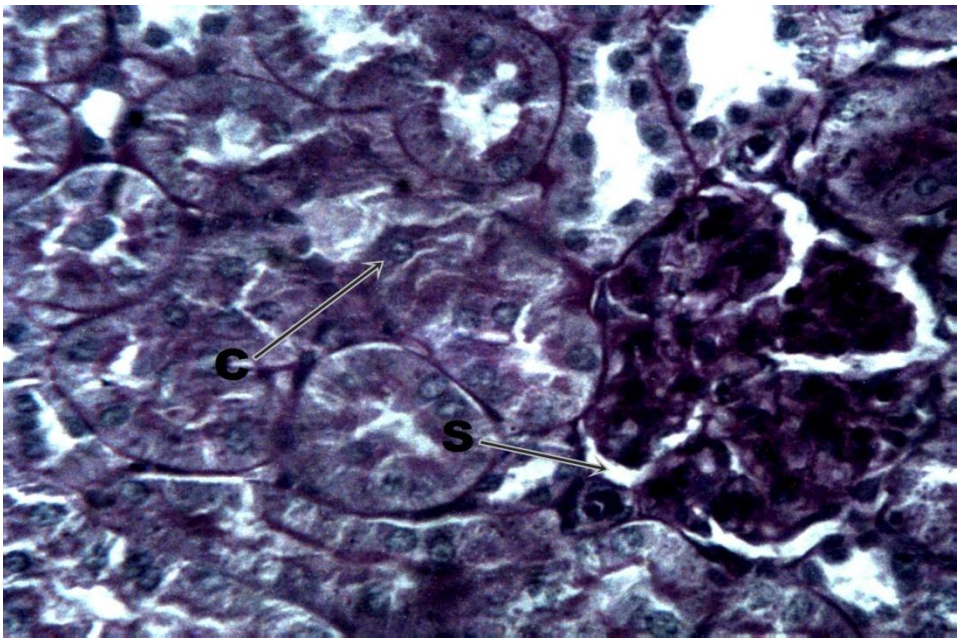


Plate 4.37: Kidney transverse section of Wistar rat of Group V demonstrating PAS positive stain intensity. Renal tubular cell (C); Urinary space (S). PAS; (Mag x400).

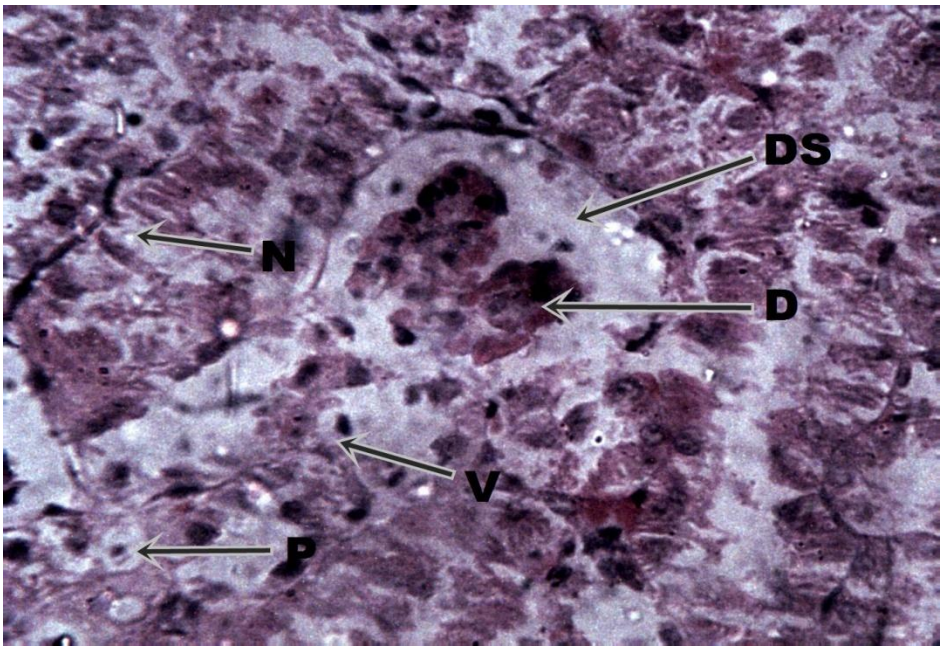


Plate 4.38: Kidney transverse section of Wistar rat of Group VI demonstrating dilated Urinary space (DS); Distorted glomerulus (D); Vacuolation (V); Pyknotic nucleus (P) and Necrosis (N). H and E, (Mag x400).

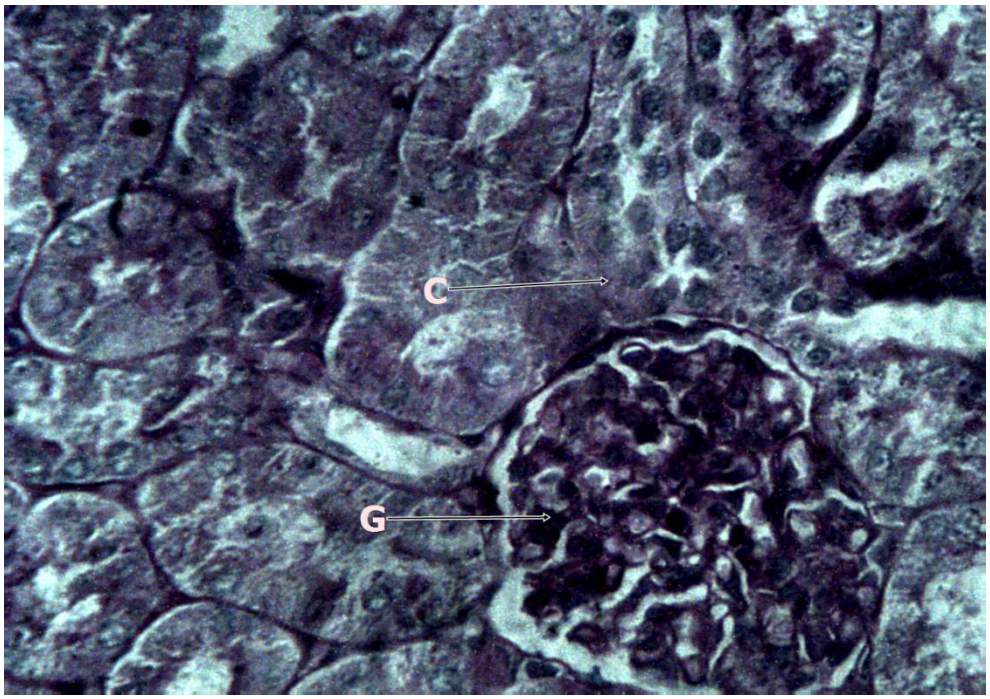


Plate 4.39: Kidney transverse section of Wistar rat of Group VI demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS stain (Mag x400).

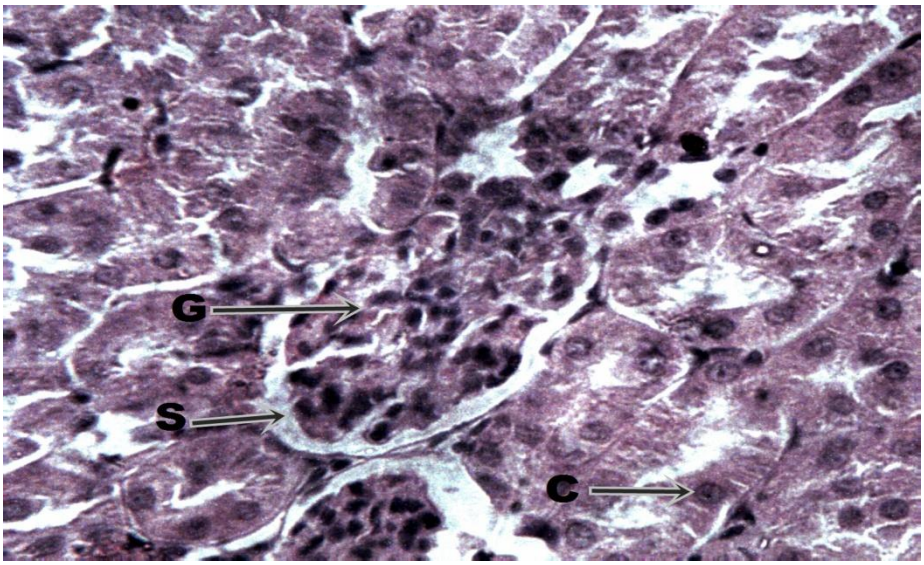


Plate 4.40: Kidney transverse section of Wistar rat of Group VII demonstrating normal histoarchitecture. Glomerulus (G); Urinary space (S); Renal tubular cell (C). H and E; (Mag x400).

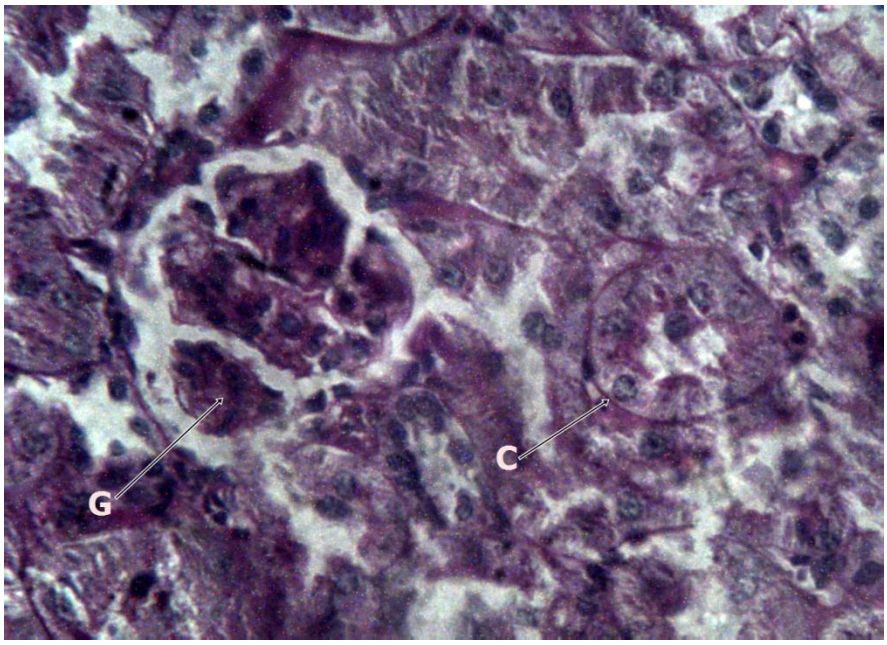


Plate 4.41: Kidney transverse section of Wistar rat of Group VII demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS stain (Mag x400).

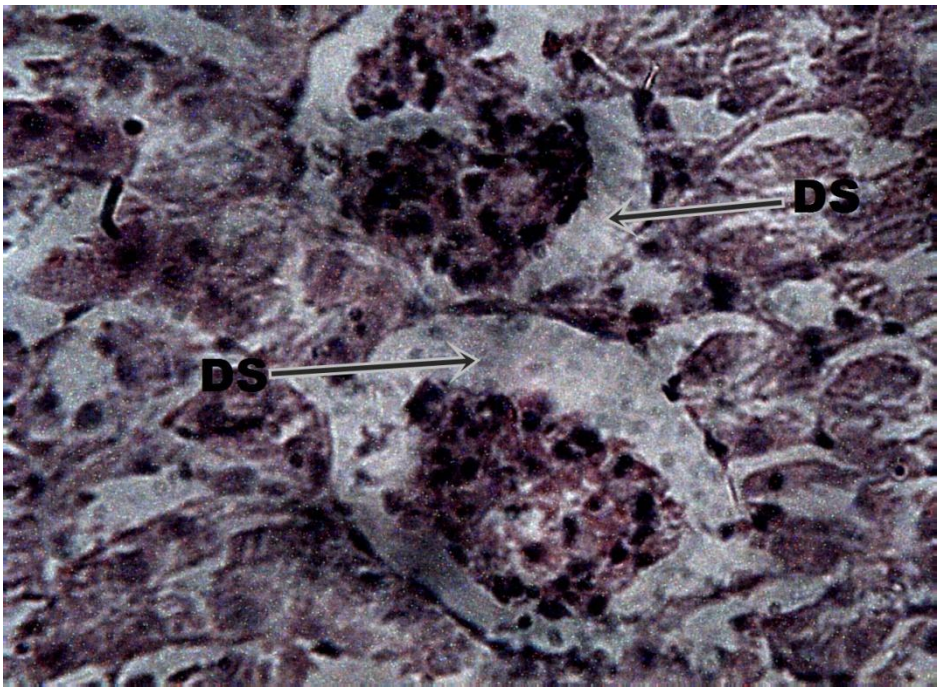


Plate 4.42: Kidney transverse section of Wistar rat of Group VIII demonstrating dilated Urinary space (DS). H and E; (Mag x400).

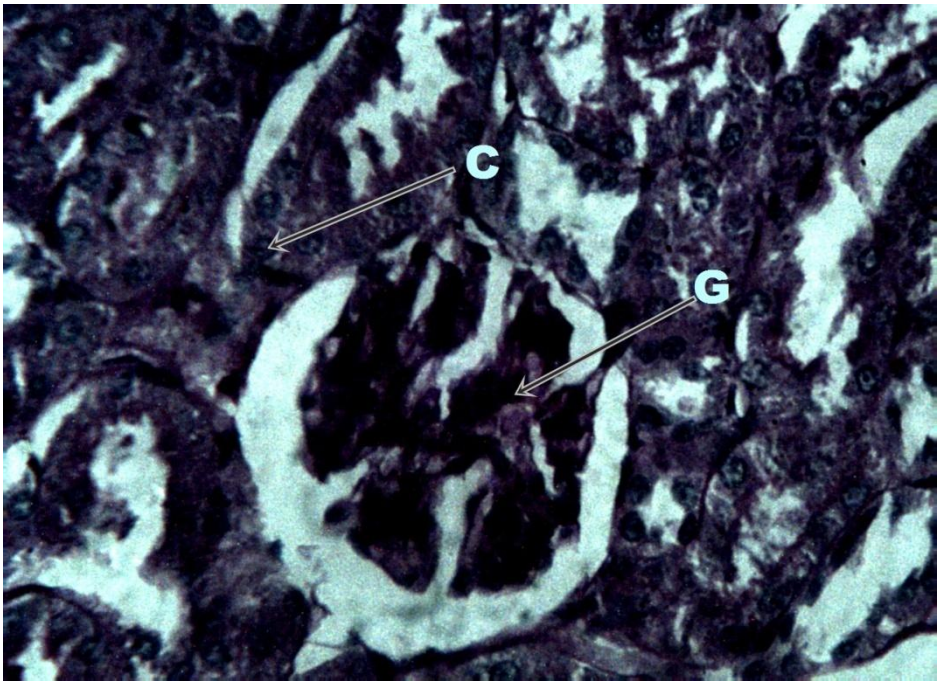


Plate 4.43: Kidney transverse section of Wistar rat of Group VIII demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS; (Mag x400).

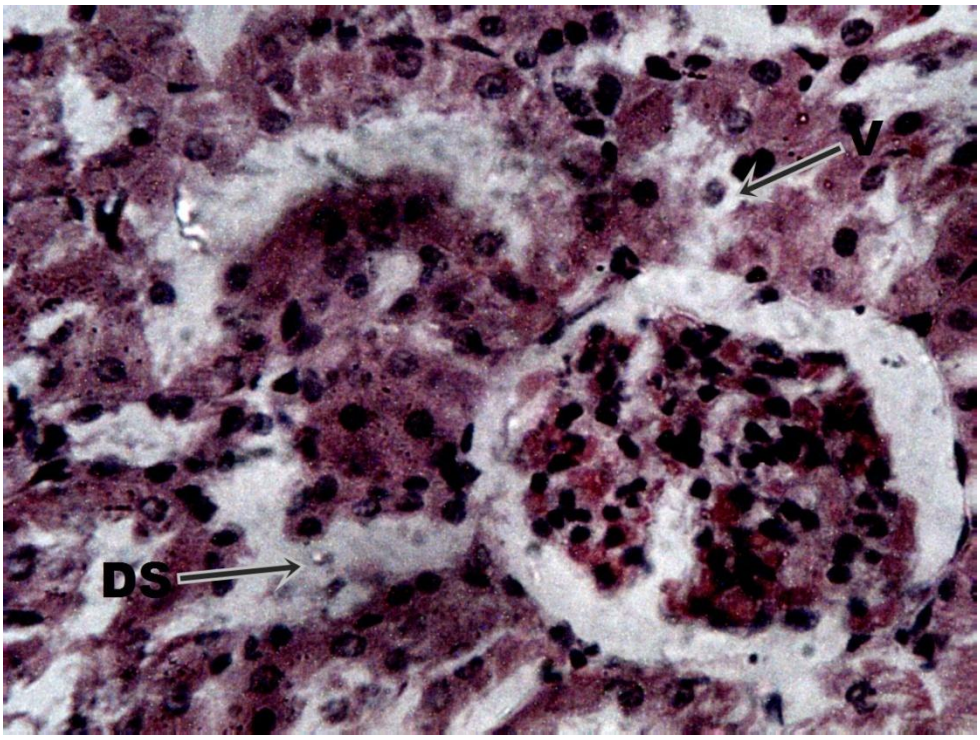


Plate 4.44: Kidney transverse section of Wistar rat of Group IX demonstrating Mild distortion. Dilated Urinary space (DS) and vacuolation (V). H and E; (Mag x400).

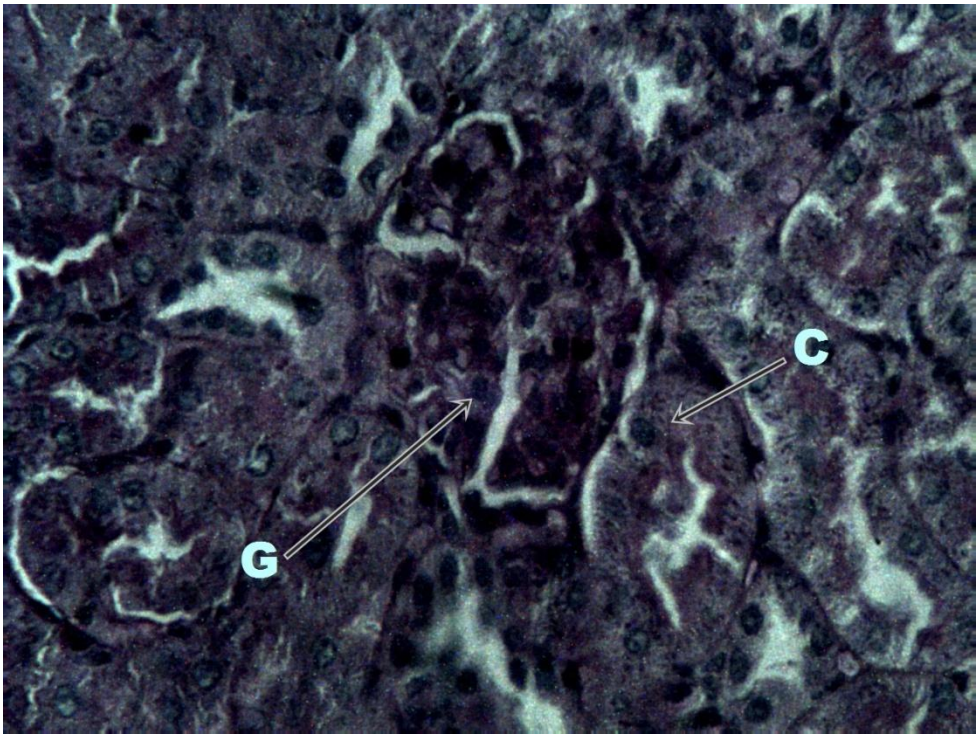


Plate 4.45: Kidney transverse section of Wistar rat of Group IX demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS; (Mag x400).

CHAPTER FIVE

5.0 Discussion

In the present study, preliminary phytochemical screening was carried out, and the protective effect of aqueous and ethanolic fruit extracts of *Phoenix dactylifera* on mercury-induced liver and kidney damages in Wistar rats were assessed using histological and histochemical techniques, biochemical investigations, lipid peroxide levels and antioxidant enzyme activities.

Phytochemical screening of the plant (*Phoenix dactylifera*) fruit extracts (aqueous and ethanol) in the present study revealed the presence of flavonoids, saponins, tannins and alkaloids which have been reported to exert hepatoprotective actions (Yoshikawa *et al.,* 2002; Chen *et al.,* 2004; Al Naqeeb *et al.,* 2009) and nephroprotective activities (Al Qarawi *et al.,* 2008; Ben Salah *et al.,* 2012) in animals and cell culture models.

Decreased physical activity exhibited by mercury-treated Wistar rats could be the result of direct treatment-related toxicity resulting in loss of appetite, improper assimilation of food and eventually weight loss. This is in concordance with reports on drug-related toxicity; altered physical activity manifesting as sluggishness and loss of appetite which indicates drug-related toxicity (Toma *et al.,* 2009; Salawu *et al.,* 2009). Body weight changes serve as a sensitive indication of the general health status of animals (Salawu *et al.,* 2009), and are used as an indicator of adverse effect of drugs and chemicals (Mukinda and Syce, 2007). Changes in the body weight of Wistar rats treated with mercury were lower than that of the extract treated groups. This is consistent with the works of Jadhav *et al.* (2007) and Wadaan (2009) who reported that the body weight gain in the mercury exposed animals lagged behind the controls.

During liver and kidney damage, cellular enzymes namely AST, ALT and ALP, BUN and serum creatinine, respectively, leaks into the blood resulting in the elevation of their serum concentration. $HgCl_2$ is known to cause liver and kidney damage leading to elevated levels of AST, ALT, ALP, BUN and serum creatinine (Ezeuko *et al.*, 2007; Shi *et al.*, 2010; Uzunhisarcikli *et al.*, 2015).

The results of the present study revealed that the rats treated with $HgCl_2$ showed increased blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and significant increase in alkaline phosphatase (ALP) as compared to the Control Group. An increase was also observed in BUN and serum creatinine levels in $HgCl_2$ treated Group. Rats that were pre-treated with silymarin, AFPD and EFPD followed by $HgCl_2$ showed a decrease in AST, ALT, ALP, BUN and Serum creatinine when compared to $HgCl_2$ treated Group. In the present study, Groups treated with only AFPD revealed decreased level of ALT and ALP but significant increase in AST level compared to $HgCl_2$ Group. Group IV, V and VII showed a remarkable significant decrease in BUN and serum creatinine levels as compared to $HgCl_2$ treated Group, while rats treated with EFPD revealed decreased level of ALT and increased levels of AST and ALP as well as decreased levels of BUN and serum creatinine levels when compared to $HgCl_2$ Group. The present result indicated that aqueous and ethanol fruit extracts of *P. dactylifera* offer protection against $HgCl_2$ hepatotoxicity and nephrotoxicity as supported by previous studies by Al-Qarawi *et al.* (2008); Saafi *et al.* (2010) and Ali *et al.* (2014).

Oral administration of $HgCl_2$ caused hepatotoxicity and nephrotoxicity as monitored by the increase in the levels of marker enzymes (transaminases, alkaline phosphatase, BUN and creatinine). In the present study, malondialdehyde (MDA) levels were increased, while the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were decreased as a result of $HgCl_2$ exposure. Pre-treatment with AFPD and EFPD followed by $HgCl_2$ decreased the level of MDA and restored

(increased) the activities of SOD, GPx and CAT in the liver and kidney. The present findings indicated that aqueous and ethanol fruit extract of *P. dactylifera* confered hepatoprotective and nephroprotective effect against $HgCl_2$ toxicity. This result agreed with previous reports on the hepatoprotective and nephroprotective effects of *P. dactylifera* by Saafi *et al.* (2010); Ali *et al.* (2014) and Uzunhisarcikli *et al.* (2015).

In the present study, light microscopic examination of routine, Haematoxylin and Eosin, and histochemical, Periodic Acid-Schiff and Gordon and Sweet stained histological sections of Wistar rats' liver and kidney tissues were done.

In the present study, liver sections of experimental animals in the Control Group showed normal histoarchitecture of the liver parenchyma (central veins, portal tract, hepatocytes and sinusoids). It has been reported that the liver is a major site of metabolism for mercury, and it accumulates in the kidneys, hence, resulting in severe damage to these organs when exposure to mercury occurs (Siham *et al*., 2008). According to Clarkson (1989); Von Burg, (1995); and Wargovich *et al.* (2001), mercury chloride is one of the most toxic forms of mercury because it easily forms organomercury complexes with proteins. Results from the present study showed necrosis of hepatocytes and dilated sinusoids as a result of mercury exposure to the experimental animals. This is in agreement with previous studies by Ribeiro *et al.* (2002); Adams *et al.* (2010) and Ibegbu *et al.* (2014). These authors reported that rats treated with $HgCl_2$ revealed necrosis of hepatocytes, congested central vein and enlarged sinusoids. The present study also demonstrated pyknotic nuclei as an indication of hepatotoxicity. This is in accordance to earlier studies revealing dark stained hepatocytic nuclei signifying pyknosis (Adams *et al.* 2010; Al Attar, 2011). Another histopathological effect of $HgCl_2$ detected in the present study was hepatocellular vacuolation supported by Singh *et al.* (2007) who, in his work reported vacuolization in the cytoplasm and reduced glycogen bodies as a result of mercury induced oxidative stress.

Histochemical results obtained in the present study revealed PAS positive staining intensity in the Control Group. Reduced PAS positive staining intensity was revealed by the group administered $HgCl_2$ as a result of reduction in glycogen bodies. The normal liver contains a large amount of glycogen; this therefore, leads to the staining of hepatocytes intensely purple with PAS stain. The PAS stain demonstrates glycogen, neutral mucosubstances and basement membranes (Saxena, 2010).

Gordon and Sweet stain revealed normal reticular framework in the liver sections obtained from the control, however, sections from the $HgCl_2$ treated group showed areas of collapsed or disintegrated reticular frame work. Reticulin fibers are thin fibers composed of type III collagen which form a delicate stromal network in many organs. The reticulin network is particularly rich in the liver and can be seen along hepatic sinusoids. Since reticulin provides the stromal support for the liver parenchyma, the reticulin stain provides important information about the architectural intergrity of the liver. When hepatocytes are damaged and undergo necrosis, the reticulin fibers surrounding them collapse in the empty space left behind (Saxena, 2010). Mild histoarchitectural distortion, such as sinusoidal dilatation and hepatocellular vacuolation observed from liver sections of Wistar rats pretreated with silymarin and extracts (AFPD *and* EFPD) followed by $HgCl_2$ indicated hepatoprotection against mercury induced hepatotoxicity.

As a hepatoprotective agent, silymarin is used for oral treatment of toxic liver damage and therapy of chronic liver diseases (Flora *et al., 1998*). Silymarin has been used for centuries to treat liver, spleen and gall bladder disorders (Shaker *et al.,* 2010). In the present study, histological features of the group pretreated with silymarin followed by $HgCl_2$ demonstrated only mild hepatocellular vacuolation when compared to $HgCl_2$ treated Group, thereby indicating hepatoprotection. These findings are in accordance

with the reports on hepatoprotection of silymarin by Dhiman and Chawla, (2005) and Song *et al.* (2006).

Treatment of Wistar rats with AFPD and EFPD followed by $HgCl_2$ showed mild sinusoidal dilatation and congested central vein compared to $HgCl_2$ treated Group. This implied that, these extracts have protective effects against mercury-induced hepatotoxicity. Results from the present study are consistent with reports on the hepatoprotective effect of *P. dactylifera* extract (Al-Qarawi *et al.,* 2004; Ahmed *et al.,* 2008). In the present study, maximum protective effects were observed in AFPD (500 and 1000mg/kgbw) treated groups, while lower protective effects were observed in EPFD (1000mg/kg) treated groups.

Treatment of Wistar rats with only AFPD and EFPD showed mild histoarchitectural distortion. Agbon *et al.* (2014) had reported that the liver sections, with aqueous fruit extract of *P. dactylifera* administration, showed mild histoarchitectural distortion such as sinusoidal dilation and congestion, which were normalized after 21 days.

Inorganic mercury accumulates primarily in the kidneys, liver, spleen, bone marrow, intestine, skin and respiratory mucosa (EUESA, 2007). In the present study, the kidney sections of experimental animals in the Control Group showed normal histoarchitecture of the kidney cortex (renal corpuscles, proximal convoluted tubule, distal convoluted tubules and blood vessels). It has been shown that kidney is the site of degradation and detoxifications of toxic substances (Rao *et al.,* 1980). It is an important organ of excretion and osmoregulation and is directly affected by heavy metal through blood circulation (Newman and Maclean, 1974). In the present study, histoarchitecture of the kidney parenchyma from mercury treated group showed severe changes when compared with those from the Control Group. These changes include dilation of Urinary space, distortion of renal corpuscle, pyknotic nuclei and necrosis. The present result was supported by the previous works of El-Shenewy and Hassan (2008) and Adams *et al.* (2010).

The present study also revealed cytoplasmic vacuolation and pyknosis in the cells of the proximal convoluted tubule as a result of nephrotoxicity by $HgCl_2$. These were in concordance with earlier studies which revealed progressive degenerative changes exhibited by kidney proximal tubules (Goering *et al., 1992*).

Mild histoarchitectural distortion, such as dilation of Urinary space, cytoplasmic vacuolation, and necrosis observed from kidney sections of Wistar rats pretreated with silymarin and extracts (AFPD *and* EFPD) followed by the administration of $HgCl_2$ indicated nephroprotection against mercury induced nephrotoxicity.

Silymarin is a poliphenolic compound extracted from *Silibum marianum* and *Cynara cardunculus* seeds and fruits. It has been demonstrated that silymarin acts as an antioxidant, reducing free radical mediated damage in tissues and inhibiting lipid peroxidation (Turgut *et al.,* 2008). In the present study, histological features of the Group pretreated with silymarin followed by $HgCl_2$ demonstrated only mild hepatocellular vacuolation when compared to $HgCl_2$ treated Group thereby indicating nephroprotection. This finding is in

accordance with the reports on nephroprotection of silymarin by (Turgut *et al.* (2008); El-Shitany *et al.* (2008).

Treatment of Wistar rats with AFPD and EFPD followed by $HgCl_2$ showed mild dilation of Urinary space, cytoplasmic vacuolation, and necrosis compared to $HgCl_2$ treated group. This implies that, these extracts have protective effects against mercury-induced nephrotoxicity. Results from the present study are consistent with reports on the hepatoprotective effect of *P. dactylifera* extract (Al-Qarawi *et al.,* 2008; Ben Salah *et al.,* 2012). In the present study, maximum protective effects were observed with AFPD (500 mg/kgbw) while lower protective effects were observed with EFPD (500mg/kg and 1000mg/kg). The

reason for this could be that the phytochemical constituents contained in AFPD are higher in quantity than in EFPD.

CHAPTER SIX

6.0 Conclusion, Recommendations and Contribution to knowledge

6.1 Conclusion

The result of the present study suggested that, the aqueous and ethanolic fruit extracts of *Phoenix dactylifera* offer protection against mercury-induced alterations in the liver and kidney of Wistar rats. The hepatoprotective and nephroprotective property of the extracts relative to the standard (silymarin), is somewhat similar, and maybe attributed to the antioxidant properties of the constituent phytochemicals, such as flavonoids. Thus, these extracts are potential candidates for use in the management and treatment of ROS-induced liver and kidney diseases.

6.2 Recommendations

Further studies such as the mechanisms responsible for the observed hepatoprotective and nephroprotective activities of aqueous and ethanolic fruit extracts of *Phoenix dactylifera*, and to determine the exact compound(s) or active ingredients responsible for its protective activity.

Further studies are required to investigate the most appropriate and efficacious extract and its dosage which best offers hepatoprotective and nephroprotective activities. In depth studies such as, ultra-microscopy, stereology and immunohistochemistry are required to elucidate the mechanism involved.

Further studies are required to investigate the possible hepatoprotective and nephroprotective activities of other solvents (e.g. organic solvents, such as methanol and chloroform) extract of *Phoenix dactylifera* fruit and other parts such as the seeds of the plant.

6.3 Contribution to knowledge

In relation to nephroprotective activity, ethanol extracts of *P. dactylifera* showed higher levels of protective efficiency.

500 mg/kg of ethanol fruit extract of *P. dactylifera* possess significant ($p<0.05$) nephroprotective activity against mercury-induced elevated kidney electrolytes. Creatinine revealed a significantly decreased level of 47.00 ± 0.00 when compared to the control 56.50 ± 7.08. Na^+ revealed a significantly decreased level of 135.00 ± 0.00 when compared to the $HgCl_2$ treated group 140.25 ± 0.25. HCO_3^- revealed a significantly decreased level of 12.00 ± 0.00 when compared to the control 19.50 ± 1.89.

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