PHARMACOLOGICAL EVALUATION OF THREE LOCALLY USED MEDICINAL PLANTS AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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B.PHARM (ABU) 2003

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JUNE, 2012

DECLARATION

I hereby declare that the work in this thesis titled "Pharmacological evaluation of three locally used medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats" was performed by me in the Department of Pharmacology and Therapeutics under the supervision of Prof. (Mrs.) O.A. Salawu and Dr. (Ms.) J.I. Ejiofor.

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

Tukur, Farida (Mrs.)	_____	June 20, 2012
Name of student	Signature	Date

CERTIFICATION

This project report/thesis/dissertation titled "Pharmacological evaluation of three locally used medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats" meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my grandmothers, “Hajiyan Layin Joji” and “Inna” who were with me at the beginning of this journey, but by Allah’s will are no longer with us. For their love, understanding and unrelenting support, may Allah grant them Al-jannah Firdaus.

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By the special grace of Allah (SWT) this project is today a success story. May He accept my humble gratitude with the best of words that He loves and approves that He be thanked with. May the everlasting peace and blessings of Allah (SWT) be upon the finality of *risala*, Muhammad (SAW), his family, companions and righteous followers.

This academic achievement would not have been possible without the scholarly guide and support of my major supervisor, Prof. O.A Salawu; and the invaluable contributions and encouragement of my minor supervisors, Dr. J.I Ejiofor and Prof. A.H Rafindadi. They have my most sincere appreciation and respect. In a similar capacity; I wish to show gratitude for the support I enjoyed from Dr. Saad, Mal. Yaro, Mal. Jamilu, Dr. Sule, Dr. Maiha, Dr. Danjuma and the entire staff of the Faculty of Pharmaceutical Sciences, as well as Mr. Tijjani and other staff of NIPRD.

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ABSTRACT

Drugs from plant and animal sources are increasingly becoming important in the therapeutic management of liver diseases. This study was undertaken to evaluate the prophylactic and curative effects of methanolic extracts of *Balanites aegyptiaca* root, *Boswellia dalzielii* stem bark, *Ipomoea asarifolia* leaves and their mixture against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Liver damage was induced by intraperitoneal injection of 0.5 ml/kg carbon tetrachloride (99.9% CCl_4) in olive oil (1:1), on alternate days, over a period of 5 days. The degree of liver damage was determined by assessing changes in hepatic indices such as serum levels of alanine transaminase, aspartate transaminase, alkaline phosphatase, triglycerides, cholesterol, total and direct bilirubin; pentobarbitone sleeping time; liver weight and histopathology. The mixture (160 mg *B. aegyptiaca*, 220 mg *B. dalzielii* and 300 mg/kg *I. asarifolia*) and each dose of the various extracts 100 mg, 200 mg and 400 mg/kg of *B. aegyptiaca*, *B. dalzielii* and *I. asarifolia* were administered orally once daily for 7 days prior to administration of CCl_4 (prophylactic study) and after the administration of CCl_4 (curative study). The degree of recovery and/or prevention of liver pathology were estimated by comparing changes in hepatic indices with that of the standard drug, silymarin (100 mg/kg). Oral LD_{50} of each extract was found to be above 2000 mg/kg (OECD-423). Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenes, sterols, resins and volatile oils in each of the extracts. Pretreatment with each extract significantly ($P<0.05$) prevented carbon tetrachloride-induced changes in hepatic indices, liver weight and histopathology compared to control. When compared to the positive control (silymarin 100 mg/kg), the protective effect of the extracts was superior and significant ($P<0.05$) at the doses of 400 mg/kg (*B. aegyptiaca*), 100 mg/kg (*B. dalzielii*) and 200 mg/kg (*I. asarifolia*). In the evaluation of the curative effect of the

various plant extracts alone (all doses of *B. aegyptiaca*, 100 mg and 200 mg/kg each of *B. dalzielii* and *I. asarifolia*) or in combination (160 mg *B. aegyptiaca*, 220 mg *B. dalzielii* and 300 mg/kg *I. asarifolia*), the extracts significantly ($P<0.05$) reversed the CCl_4 -induced liver pathology as seen in the values of the hepatic indices, liver weight and histopathology. This effect was comparable with that of silymarin. Prophylactic treatment with *B. aegyptiaca*, *B. dalzielii* and the mixture produced significant increases in pentobarbitone sleeping time while *I. asarifolia* decreased the sleeping time when compared to the control. Curative studies revealed a significant ($P<0.05$) decrease in sleeping time following treatment with all the extracts. These results indicate that methanolic extracts of *B. aegyptiaca*, *B. dalzielii* and *I. asarifolia* are relatively safe orally and possess potent hepatoprotective properties that may be useful in the management of liver diseases.

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ABBREVIATIONS

ABU	-	Ahmadu Bello University
AFP	-	α-Fetoprotein
ALB	-	Albumin
ALP	-	Alkaline phosphatase
ALT	-	Alanine transaminase
ANOVA	-	One Way Analysis of Variance
AST	-	Aspartate transaminase
B.A	-	*Balanites aegyptiaca*
B.D	-	*Boswellia dalzielii*
b.wt	-	Body Weight
BLT	-	British Liver Trust
CAR	-	Constitutive Androstane Receptor
CBC	-	Complete Blood Count
CCl_3	-	Trichloromethyl
CCl_3OO	-	Trichloromethyl peroxy
CCl_4	-	Carbon tetrachloride
CHOL	-	Cholesterol
$Cl_3C\text{-}CCl_3$	-	Hexachloroethane
CNS	-	Central Nervous System
$COCl_2$	-	Phosgene
CT	-	Computed Tomography
DBIL	-	Direct Bilirubin
DNA	-	Deoxyribonucleic acid
EASL	-	European Association for the Study of the Liver
ECDC	-	Epidemiological Report on communicable Diseases

ED_{50}	-	Median Effective Dose
FAO	-	Food and Agriculture Organisation
GABA	-	Gamma-amino butyric acid
HAV	-	Hepatitis A virus
HBsAg	-	Hepatitis B surface antigen
HBsAg	-	Hepatitis B surface antigen
HBV	-	Hepatitis B virus
HCC	-	Hepatocellular Carcinoma
HCV	-	Hepatitis C virus
HDV	-	Hepatitis D virus
HEV	-	Hepatitis E virus
I.A	-	*Ipomoea asarifolia*
IALM	-	*Ipomoea asarifolia* leaf meal
IgA	-	Immunoglobulin A
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
INF-α	-	Interferon α
INF-α2a	-	Interferon α2a
INR	-	International Normalized Ratio
LCAT	-	Lecithin Cholesterol Acyl transferase
LD_{50}	-	Median Lethal Dose
LDH	-	Lactate dehydrogenase
LW	-	Liver Weight
MDME	-	Microsomal Drug Metabolizing Enzymes
MRI	-	Magnetic Resonance Imaging
NAFLD	-	Non-alcoholic Fatty Liver Disease

NASH	-	Non-alcoholic Steatohepatitis
NIPRD	-	National Institute for Pharmaceutical Research and Development
OECD	-	Organisation for Economic Co-operation and Development
MIX	-	A mixture of *B. aegyptiaca*, *B. dalzielli* and *I. asarifolia*
PST	-	Pentobarbitone Sleeping Time
PT	-	Prothrombin Time
RNA	-	Ribonucleic acid
SEM	-	Standard Error of the Mean
SGOT	-	Serum glutamate oxaloacetate transaminase
SGPT	-	Serum glutamate pyruvate transaminase
TBIL	-	Total Bilirubin
TRIG	-	Triglycerides
UK	-	United Kingdom
VLDL	-	Very Low Density Lipoprotein
WHO	-	World Health Organisation
WHO/PRO	-	World Health Organisation regional Office for the Pacific

CHAPTER ONE

1.0 INTRODUCTION

1.1 Statement of Problems

Liver disease (also referred to as hepatic disease) is a term that describes significant pathological changes in the anatomy and functions of the liver. There are different types of liver diseases, usually classified based on their causes. The most prevalent forms of the disease are; hepatitis, typically caused by viruses, but also by drugs, poisons, injury, autoimmune processes or genetic anomalies (Hemalatha, 2008) and alcoholic liver disease, caused by excessive alcohol consumption. Other common hepatic diseases like cirrhosis, fatty liver disease and liver cancer may also result from the above mentioned causes.

A normal working liver is crucial for survival because of its major role in drug metabolism, glycogen storage, bile production, plasma protein synthesis and a host of other functions. The large size and strategic location of the liver, together with its diverse functions make it vulnerable to constant attack by toxic substances which can cause injury or disease. To overcome this, the liver has been endowed with an amazing ability to naturally regenerate lost tissue in a bid to maintaining a balance between cell death and regeneration. However, if an injury or disease progresses beyond the liver tissue's capacity to regenerate new cells, an imbalance occurs, leading to hepatic injury and subsequently liver failure (Owais *et al.*, 2006).

Diseases of the liver may be acute, where damage occurs suddenly, or chronic, where the damage develops slowly over a period of time. Though chronic liver disease is much more common than acute (McLaughlin, 2000), both are associated with

remarkably high morbidity and mortality rates, especially in developing countries where health care facilities are grossly inadequate. Undiagnosed or poorly managed liver diseases may eventually affect the entire body system, causing other illnesses like kidney disease, anaemia, encephalopathy, gastrointestinal bleeding and peptic ulcers (McLaughlin, 2000).

Despite the tremendous medical advancement observed globally in the last decade, liver disease still remains one of the major causes of death in the world, creating a serious public health concern. In the European Union, the prevalence of liver diseases is estimated to be approximately 6% (EASL, 2012) translating to about 29 million people, with hepatitis, the most common liver disease affecting over 10 million of this population. Reports also show a greater increase in hepatitis C cases, estimated at 8.7 per 100,000 compared to that of hepatitis B at 1.5 per 100,000 (ECDC Epidemiological Report on communicable Diseases, 2007). Another survey stated that 16,087 people in the UK died from liver disease in 2008 (British Liver Trust, 2009), a 4.5% increase compared to 2007. With reports from several parts of the world indicating a rise in liver problems, the British Liver Trust (BLT) estimates that the disease could kill more than 60,000 people in the next decade (Patel *et al.*, 2008). In the same vein, the National Vital Statistics Report for the United States revealed that chronic liver disease and cirrhosis were responsible for 31,802 deaths in 2010 i.e. 10.3 out of 100,000 of the population (Murphy *et al.*, 2012).

In Nigeria and some parts of Africa, data representing the burden of the disease is grossly inadequate due to a large number of reasons. Nonetheless, the prevalence of some common types of hepatic diseases has been documented. Hepatitis B surface

antigen was reported to have a prevalence ranging between15–20% and up to 30% in some parts of Nigeria (Amazigo and Chime, 1990; Bojuwoye, 1997; Onyekwere *et al.*, 2002), while that of Hepatitis C is estimated between 2.9-10.3% (Lesi and Kehinde, 2003; Koate *et al.*, 2005; Ejele *et al.*, 2006). Hepatocellular carcinoma (HCC) alone, the most common cause of cancer mortality in middle-aged and elderly Nigerians (Ndububa *et al.*, 2001) accounted for 42.5% of deaths from liver disease, while the complications of cirrhosis accounted for 21.1% of the deaths in a study carried out in 1997 (Ladep and Taylor-Robinson, 2007).

In spite of these alarming statistics, modern medicine is still unable to provide effective therapeutic agents for the treatment of hepatic diseases. The available orthodox remedies such as corticosteroids, interferon, penicillamine, antiviral and immune suppressant drugs only provide symptomatic relief, without significant changes to the progression of the disease (Owais *et al.*, 2006). In addition, these drugs are not only expensive, but saddled with high relapse rates and severe side effects some of which are, ironically, liver related e.g. azathioprine (an immunosuppressant) can cause cholestatic jaundice (Ramagnuolo *et al.*, 1998) while virazole (an antiviral drug) can cause elevation of serum transaminase (Cattral *et al.*, 1999). Quite a number of orthodox medicines used in the treatment of other disease conditions like cancer, tuberculosis and epilepsy are mildly hepatotoxic and with no suitable alternatives available, they have to be used for a long time, predisposing the patients to chronic liver disease.

In view of these challenges, it has become imperative to provide affordable, safe and effective therapeutic agents to replace the existing drugs used for the management of

hepatic diseases. In an endeavor to reduce the incidence and prevalence of liver diseases, minimize the risks of adverse effects associated with the current treatment modalities and provide a global solution to the immense burden of the disease, this study seeks to substantiate purported traditional claims of the effectiveness of some local plants in the treatment of liver conditions.

1.2 Justification

Diseases that affect the liver primarily interfere with liver function, leading to a decline in its activities. During the early stages of liver disease, symptoms are sometimes vague (e.g. fatigue, mild weight loss and loss of appetite) or even completely absent, possibly due to the inherent regenerative ability of the liver and its large reserve capacity. In most cases, symptoms begin to appear only after extensive damage has occurred. Symptoms of acute liver disease usually manifest as jaundice, dark urine, light stools, loss of appetite, nausea, vomiting, and diarrhea; while those of chronic liver disease may include abdominal swelling (due to ascites), unexplained weight loss or gain, and abdominal pain.

The treatment for liver diseases though different, depending on the type, usually focuses on the relief of symptoms and complications. In addition, withdrawal of the causative hepatotoxin generally reverses its effects. However, since in most cases the causative agent is unknown, prevention of exposure becomes impossible. In some of the few cases where the causative agent is known, withdrawal may not be feasible, such as during treatment with anticancer drugs, which are mildly hepatotoxic (Owais *et al.*, 2006). Undoubtedly, this poses an overwhelming need for alternative medications effective in the treatment of a variety of liver diseases.

Current researches in many parts of the world centre on the use of medicinal plants as liver protective drug sources (Aly *et al.*, 2004; Agarwal *et al.*, 2006; Kyung *et al.*, 2007). Several plants reported to possess hepatoprotective activity have been scientifically investigated to identify the phytochemical constituents responsible for this activity and ascertain their effectiveness. Examples of such plants include; *Picrorrhiza kurroa* (Saraswat *et al.*, 1997; Anssari *et al.*, 1998), *Andrographis paniculate* (Handa, 1993) *Eclipta alba* (Saxena, 1993) and *Phyllanthus emblica* (Gulati, 1994).

This trend has been guided by the fact that many people in developing countries rely on traditional healing practices and medicinal plants (WHO/PRO, 1998), because it is the most affordable and accessible system of health care. Most importantly, long historical use has demonstrated the relative safety and efficacy of traditional medicine (WHO, 2000). Even with the great potentials of herbal medicine, it is important to corroborate traditional medical knowledge with detailed scientific findings, to provide evidence of safety and efficacy of purported beneficial plants (WHO, 2002).

1.3 Theoretical Framework

Hepatocytes, the most predominant type of liver cells are responsible for most of the numerous functions carried out by the liver such as synthesis, metabolism, transport and storage. They also contain a host of enzymes like aspartate transaminase (AST), alanine transaminase (ALT) etc. ALT is found predominantly in the liver while AST also exists in the heart, skeletal muscle, kidneys, and pancreas in addition to the liver. In hepatic injury, the transport function of the cells is impaired, leading to a leakage of these enzymes in serum (Usha *et al.*, 2008). This increase in serum transaminase levels

is a clear indication of hepatocellular damage and loss of the functional integrity of its cell membrane.

Other markers of hepatic damage include increased serum level of alkaline phosphatase (ALP) and decreased total protein and albumin levels in the blood. Physically, the first clear sign of liver injury usually results from increased levels of serum bilirubin, a final product of haeme degradation. It presents as dark colored urine (bilirubinuria) and a condition known as jaundice (hyperbilirubinaemia), which is characterized by yellowish discoloration of the skin, conjunctival membranes and other mucous membranes. Hyperbilirubinaemia may be due to one or more of the following; increased bilirubin production, decreased bilirubin uptake by the liver (conjugation) and decreased biliary excretion (Eldon, 2009a). The presence of jaundice therefore, is a cardinal feature of liver disease usually showing a disturbance within the hepatobiliary system.

In local communities within Zaria Local Government of Kaduna State, a concoction of several medicinal plants is administered to persons presenting with signs of jaundice. The concoction is usually made up of three plants (*Boswellia dalzielii* stem bark, *Ipomoea asarifolia* leaves, and *Balanites aegyptiaca* root), though sometimes other plants may be added to the mixture. The plants are usually washed and boiled together in clean water for about 15 mins and left to soak overnight, after which the mixture is strained and the resulting extract administered as a medicine. The concoction is locally believed to cleanse the system of the yellowing substance (bilirubin), probably by decreasing its production, enhancing its metabolism and/or excretion.

This research intends to evaluate the hepatoprotective activity and safety of these three plants i.e. *Boswellia dalzielii, Ipomoea asarifolia* and *Balanites aegyptiaca*, both individually and together as a mixture. However, to validate the use of these medicinal plants in the management of liver diseases, an adequate follow-up scientific research has to be carried out to isolate and characterise the active constituents as well as the possible mechanisms of their action. A series of clinical trials would also have to be conducted before the standardized drugs are made available to the public.

The presence or absence of hepatoprotective activity of the plants will be assessed using the first-line testing parameters often measured in patients presenting with liver diseases i.e. determination of urinary and serum levels of bilirubin (total, indirect and direct fractions), serum AST/ serum glutamate oxaloacetate transaminase (SGOT), serum ALT/ serum glutamate pyruvate transaminase (SGPT), serum levels of albumin, total protein and alkaline phosphatase (Kobos and Roche, 2004). Other tests may comprise determination of serum cholesterol and triglyceride levels.

An increase in unconjugated or indirect bilirubin fraction (> 85%) reflects increased bilirubin production or defective liver conjugative uptake. Reduced serum albumin often tends to worsen bilirubin accumulation, because albumin normally binds bilirubin in a ratio of 1:1 at the primary high affinity binding site (Hansen, 2007). In clinical situations, depending on the results of these initial tests, further serum tests (such as serum albumin) or imaging studies may be warranted. Increase in conjugated or direct bilirubin fraction (> 50%) results from decreased bile formation or excretion (cholestasis). In markedly elevated blood levels, conjugated bilirubin spills into urine (bilirubinuria), but elevated levels of unconjugated bilirubin is not excreted in urine

because it is both highly bound to albumin and water insoluble. The development of severe hyperbilirubinemia in primary biliary cirrhosis, alcoholic hepatitis, and acute liver failure suggests a poor prognosis (Eldon, 2009a).

Conditions of acute liver injuries due to ischemia or drugs like acetaminophen usually result in a rise greater than 10,000 units of ALT per litre (Johnston, 1999). In conditions of acute alcoholic hepatitis, levels of AST and ALT rise to several hundred units per liter, where the ratio of AST to ALT is usually greater than 1, as against hepatitis caused by infections where the rise in ALT is more than that of AST (Cohen and Kaplan, 1979). The assessment of transaminase levels (AST and ALT) may be less helpful in chronic liver diseases because levels can be normal or only slightly elevated when there is little liver parenchyma left to damage.

Alkaline phosphatase and glutamyltransferase are markers for cholestasis. Bile obstruction results in a rise above the normal levels for these two enzymes (Johnston, 1999).

1.4 Aim and Objectives of the Study

The main aim of this study is to investigate the hepatoprotective properties of three plants (*Balanites aegyptiaca* root, *Boswellia dalzielii* stem bark, and *Ipomoea asarifolia* leaves) traditionally used for the treatment of liver diseases in Zaria. Specific objectives for the study are:

1. To evaluate the methanolic extract of each plant for hepatoprotective activity
2. To evaluate the hepatoprotective activity of a mixture of the methanolic extracts of the three plants

3. To characterize the phytochemical constituents present in each plant extract

1.5 Hypothesis

The null hypothesis governing this study is that the methanolic extracts of *Balanites aegyptiaca* root, *Boswellia dalzielii* stem bark and *Ipomoea asarifolia* leaves, alone or in combination do not possess hepatoprotective activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Liver

The liver is the largest organ in the human body weighing approximately 1.8 kg in men and 1.3 kg in women (British Liver Trust, 2011). It is found in all vertebrates and some invertebrates as well. In humans, the liver is located in the upper right quadrant of the abdominal cavity, just below the diaphragm, overlying the gallbladder. It is wedge-shaped, dark reddish brown in colour and divided into 4 lobes; right, left, caudate, and quadrate. The right and left lobes are the largest, while the caudate and quadrate are smaller and located posteriorly (Eric, 2002). These lobes are further subdivided into approximately 100,000 small lobules.

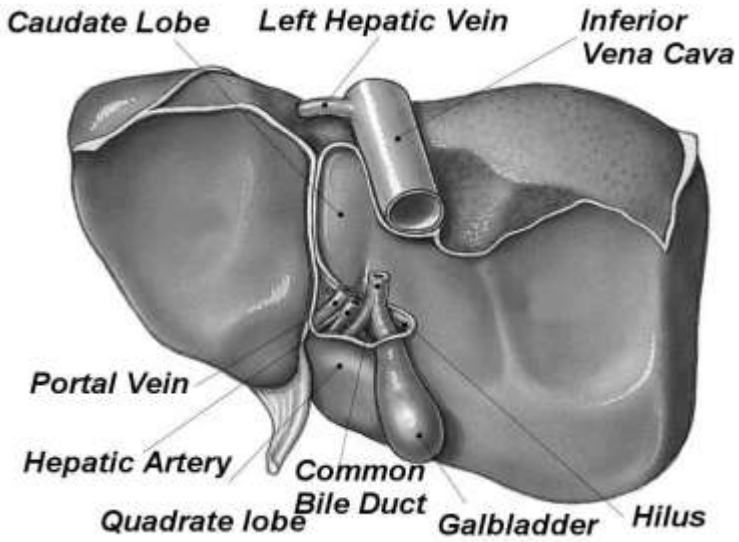


Figure 2.1: Structure of the Liver

The hepatic portal vein and hepatic arteries are responsible for approximately 75% of the liver's blood supply. The portal vein carries nutrient-filled venous blood at low pressure from the spleen, gastrointestinal tract, pancreas and gallbladder, while the

arteries supply high-pressured arterial blood from the heart (aorta). Portal venous blood is low in oxygen, since the capillary bed of the gastrointestinal tract extracts most of the oxygen. Arterial blood however, originates directly from the aorta and is thus saturated with oxygen. Blood flows through the sinusoids (canals formed by the plates of liver cells) and empties into a central vein found in each lobule. The central veins coalesce into hepatic veins, which carry deoxygenated blood back to the heart via the inferior vena cava.

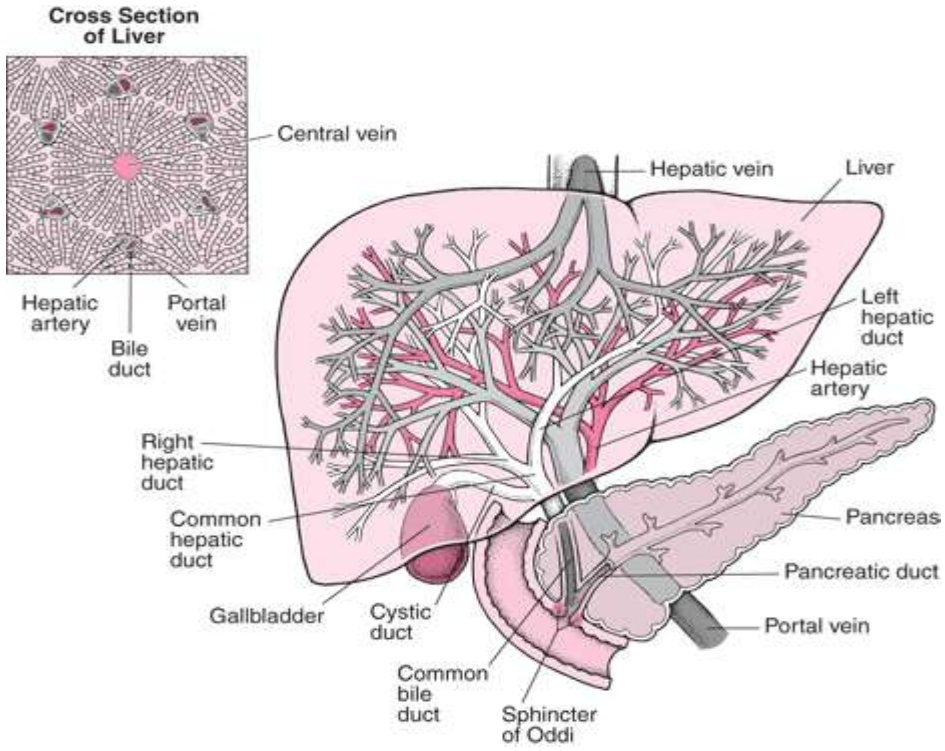


Figure 2.2: Sectional view of the liver, pancreas and gall bladder

The liver is made up of five (5) cell types, namely; hepatocytes, Kupffer cells, sinusoidal endothelial cells, bile duct epithelial cells, and Ito cells (Eric, 2002). Hepatocytes represent 60% of the liver's cells and are responsible for most of its synthetic and metabolic activities. There are approximately 202,000 hepatocytes in every milligram of liver tissue, with each having an average lifespan of 150 days (British Liver Trust, 2011).

Kupffer cells are macrophages that reside in the sinusoids. They break down heme (the iron-containing pigment in hemoglobin) into bilirubin, one of the chief pigments of bile and get rid of old red blood cells and bacteria (Eric, 2002). Sinusoidal endothelial cells have large pores that allow most proteins and other substances to pass freely to and from the liver. Bile duct epithelial cells line the interlobular bile ducts within the portal triads, while Ito cells transform into cells that produce collagen in the presence of liver injury (Eric, 2002).

2.2 Functions of the Liver

The liver has been estimated to have over 500 functions. These can be broadly classified into three (3):

2.2.1 Synthesis

The liver is responsible for the synthesis of a variety of proteins such as albumin, coagulation factors, alpha1-antitrypsin, very low density lipoprotein, insulin-like growth factor, thrombopoietin etc (Eric, 2002). It also produces glucose from certain amino acids, lactate or glycerol (gluconeogenesis), glycogen from glucose (glycogenesis), cholesterol and triglycerides (lipogenesis). In the first trimester foetus, the liver serves as the major site of red blood cell production (Wikepedia, 2011). In addition, the liver synthesizes bile, a greenish liquid that is required for the emulsification of fat i.e. it breaks fats down into smaller components so they can be digested in the small intestine (Eric, 2002).

2.2.2 Metabolism and elimination

Lipophilic substances (e.g. drugs) are metabolized by hepatocyte enzymes (cytochrome P-450 enzymes) in the liver to either less/non toxic substances (detoxification) or more

toxic substances (toxification). Such substances are bound to albumin and converted to more water soluble compounds that can be readily excreted in bile or urine. Failure of these metabolic functions represents the basic pathophysiology of all forms of liver disease.

2.2.3 Storage

The liver balances the supply of nutrients with the body's demand. It stores glucose as glycogen, and converts it back to glucose when the body is in need of glucose; it also converts excess glucose and amino acids into fatty acids for storage (Eric, 2002). The liver stores vitamin A (1-2 years' supply), vitamin D (1-4 months' supply), vitamin B_{12}, iron and copper.

2.3 Liver Diseases

Liver diseases are referred to as conditions, disorders, and infections that affect the cells, tissues, structures or functions of the liver, resulting in mild or severe damage (McLaughlin, 2000). Diseases of the liver could be acute, sub-acute or chronic. Acute liver diseases occur suddenly within a period of less than 3 months, the sub-acute type lasts for 3-6 months, while chronic liver diseases usually last for more than 6 months (Eric, 2002). Chronic liver disease rates are twice as high in men compared to women and the disease is more prevalent than acute liver disease (McLaughlin, 2000).

2.3.1 Types of liver disease

There are over 100 known forms of liver diseases caused by a variety of factors and affecting everyone from infants to older adults (Canadian Liver Foundation, 2011). The most common types of liver diseases are hepatitis, alcoholic liver disease, cirrhosis,

hemochromatosis, fatty liver disease and liver cancer. Others include Wilson's disease, primary sclerosing cholangitis, primary biliary cirrhosis, Budd-Chiari syndrome and Gilbert's syndrome. These diseases occur as a result of exposure to one or more of the following; alcohol, toxins in the environment, food contaminants, certain drugs and medicinal herbs. Other causes are metabolic disorders, viral infections, autoimmune disorders, congenital birth defects, nutritional deficiencies and trauma to the liver. In some cases however, the exact cause of liver injury is not known.

2.3.1.1 Hepatitis

Hepatitis, also known as "inflammation of the liver" is the most common type of liver disease, characterized by diffuse or patchy necrosis, which could be acute or chronic. Hepatitis has several causes, the major ones being specific hepatotropic viruses (hepatitis A, B, C, D and E viruses), alcohol, and drugs. The less common causes include other viral infections (e.g. infectious mononucleosis, yellow fever, cytomegalovirus infection) and leptospirosis. Various systemic infections and other illnesses may produce small focal areas of hepatic inflammation or necrosis that is usually asymptomatic (Cohen, 2007).

I. Acute viral hepatitis is diffuse liver inflammation caused by specific hepatotropic viruses that have diverse modes of transmission and epidemiology (Cohen, 2007). The individual prevalence for each virus varies based on geographical location and risk factors. Most cases of acute viral hepatitis are self limiting, resolving spontaneously 4 to 8 weeks after symptom onset, some however progress to chronic hepatitis, while some rare cases progress to acute hepatic failure, known as fulminant hepatitis.

a. Hepatitis A virus (HAV) is the most frequent cause of acute viral hepatitis and is particularly common among children and young adults. It is spread primarily by fecal-oral contact, thus occurring in areas of poor hygiene. HAV could also be waterborne, food-borne, acquired from contaminated raw shellfish and sometimes through person-to-person contact.

b. Hepatitis B virus (HBV) is the second most common cause of acute viral hepatitis. It is usually transmitted parenterally through contaminated blood or blood products. Routine screening of donor blood for hepatitis B surface antigen (HBsAg) has drastically reduced the previously common post-transfusion transmission, but transmission through needles shared by drug users, contact with other body fluids and vertical transmission from mother to child remain common (Cohen, 2007). Many cases of acute hepatitis B occur sporadically without a known source. Hospital personnel who come in contact with blood and patients in renal dialysis and oncology units are at high risk for HBV infection.

c. Hepatitis C virus (HCV) infection is another major cause of acute viral hepatitis. It is mostly transmitted in blood through sharing of drug needles, tattoos and body piercing. Like HBV, transmission through blood transfusion has now become rare, but unlike HBV, sexual transmission and transmission from mother to infant of HCV are relatively uncommon. Some sporadic cases occur in patients without apparent risk factors (Cohen, 2007).

d. Hepatitis D virus (HDV) or delta agent, is a defective RNA virus that can replicate only in the presence of HBV. It occurs uncommonly as a co-infection with acute hepatitis B or as a super-infection in chronic hepatitis B (Cohen, 2007).

e. Hepatitis E virus (HEV) is an enterically transmitted RNA virus. Infections may be waterborne or sporadic. Outbreaks of acute HEV infection linked to fecal

contamination of the water supply have occurred in China, India, Mexico, Pakistan, Peru, Russia, and Central and Northern Africa (Cohen, 2007).

II. Chronic hepatitis is commonly caused by hepatitis B and C viruses, autoimmune mechanisms (autoimmune hepatitis), and drugs. 5 to 10% of cases of HBV infection, with or without hepatitis D virus (HDV) co-infection, and about 75% of cases of HCV infection become chronic, many cases are idiopathic (Cohen,2007). Drugs such as isoniazid, methyldopa, nitrofurantoin and rarely acetaminophen can cause chronic hepatitis. Other causes of chronic hepatitis include alcoholic hepatitis, nonalcoholic steatohepatitis, α1-antitrypsin deficiency and Wilson's disease.

2.3.1.2 Cirrhosis

Cirrhosis is a widespread and progressive chronic liver condition in which hepatocyte activity is depressed due to excessive amounts of fibrous scar tissue which inhibit blood flow and destroy the liver's internal structure, thereby impairing its functions and regenerative ability (Eric, 2002). The obstruction in blood flow can cause portal hypertension leading to additional complications such as ascites, shunting of veins around the liver and swollen veins in the esophagus (varices). Other potential complications of cirrhosis include bleeding problems, hepatic encephalopathy, kidney disorders, osteoporosis, and liver cancer.

Cirrhosis is the late stage of hepatic fibrosis and a leading cause of death worldwide. In developed countries, most cases result from chronic alcohol abuse or chronic hepatitis C, while the cause in developing and under developed countries is usually chronic hepatitis B (Eldon, 2009b). Cirrhosis may also result from mechanical bile duct

obstruction, primary biliary cirrhosis and primary sclerosing cholangitis. Cirrhosis of unknown etiology (cryptogenic cirrhosis) is not very common.

2.3.1.3 Alcoholic Liver Disease

This refers to a range of conditions and related symptoms that develop when the liver becomes damaged due to excessive alcohol intake. Alcohol consumption is high in many parts of the world, especially the Western countries. In the United States for example, more than 10% of the population either abuse or are dependent on alcohol and males constitute two-thirds of this group (Eldon, 2009c). Alcoholic liver disease occurs in three different variants, occurring individually or together in the same patient (Eric, 2002). These variants have overlapping features and usually present in the following sequence: fatty liver, alcoholic hepatitis, then cirrhosis. Hepatocellular carcinoma may also develop, especially in association with iron accumulation (Eldon, 2009c).

Fatty liver (steatosis) is the initial and most common consequence of excessive alcohol consumption. It is a potentially reversible condition characterized by hepatomegaly. Alcoholic hepatitis (steatohepatitis) is a combination of fatty liver, diffuse liver inflammation, and liver necrosis at varying degrees of severity. Alcoholic cirrhosis, on the other hand, is an advanced form of the disease characterized by extensive fibrosis that disrupts normal liver architecture and relatively small nodules (micronodular cirrhosis) causing the liver to shrink (Eldon, 2009c).

Manifestation of alcoholic liver disease is largely dependent on a number of risk factors, they are:

a. Quantity and duration of alcohol use: Generally, there is a linear correlation between the amount and duration of alcohol use and the development of liver disease in susceptible people. For men, the risk increases markedly for those who consume > 40 g of alcohol/day for more than 10 years. Cirrhosis usually develops when consumption exceeds 80 g/day for more than 10 years (Eldon, 2009c).

b. Sex: Women are more susceptible to alcoholic liver disease, as they require only 20 to 40 g/day of alcohol to be at risk, also because they have less alcohol dehydrogenase in their gastric mucosa, thereby reducing first-pass oxidation of alcohol (Eldon, 2009c).

c. Genetic and metabolic traits: Alcoholic liver disease often runs in families, suggesting genetic factors such as deficiency of cytoplasmic enzymes that eliminate alcohol (Eldon, 2009c).

d. Nutritional status: Obesity and diets high in unsaturated fat and low in protein increase susceptibility.

Other risk factors include concomitant hepatitis C disease which occurs in more than 25% of alcoholics, worsening the progression of liver disease; and iron accumulation in the liver, which may or may not be related to iron intake.

2.3.1.4 Fatty Liver Disease

Fatty liver (hepatic steatosis) disease is the excessive accumulation of lipid in hepatocytes, the most common liver response to injury. The disease could result from excessive alcohol consumption or non-alcoholic causes i.e. non-alcoholic fatty liver disease (NAFLD). NAFLD includes simple fatty infiltration (a benign condition) and nonalcoholic steatohepatitis, a less common but more important variant (Herrine, 2009).

Nonalcoholic steatohepatitis (NASH) is the most common chronic hepatitis not caused by viruses. In most cases, it develops in patients with one or more of the following risk factors: obesity, dyslipidemia, and glucose intolerance. Though the disease can occur in all age groups, it is typically found in patients between the ages of 40-60. Advanced NASH can result in cirrhosis and portal hypertension (Herrine, 2009).

2.3.1.5 Hepatocellular Carcinoma

Hepatocellular carcinoma (hepatoma) is the most common type of primary liver cancer that results in about 14,000 deaths annually in the United States and even more common in East Asia and sub-Saharan Africa (Herrine, 2007). Other less common primary liver cancers include fibrolamellar carcinoma, cholangiocarcinoma, hepatoblastoma and angiosarcoma. Hepatocellular carcinoma usually occurs in patients with cirrhosis and in areas where infections with HBV and HCV are prevalent. Other causes of hepatocellular carcinoma are hemochromatosis and alcoholic cirrhosis. Environmental carcinogens such as fungal aflatoxins are believed to contribute to the high incidence of hepatocellular carcinoma in subtropical regions (Herrine, 2007).

2.3.2 Symptoms of liver disease

Symptoms of liver disease may vary depending on the type and extent of the disease. In many cases, especially at the early stages of the disease, there may be no symptoms. Sometimes the disease presents with symptoms that are vague, like weakness and loss of energy. In acute liver disease, symptoms may include jaundice, dark urine, light stools, loss of appetite, nausea, vomiting, and diarrhea. Chronic liver disease typically presents with abdominal swelling (due to ascites), pruritus, unexplained weight loss or gain, and abdominal pain. Common liver disease symptoms include:

2.3.2.1 Jaundice

This is usually the first sign, and sometimes the only sign, of liver disease. It manifests as a yellow discoloration of the skin and sclera due to abnormally high levels of bilirubin (bile pigment) in the blood. Urine is usually dark because of the bilirubin excreted through the kidneys. Increase in bilirubin levels may be as a result of inflammation, other abnormalities of the liver cells, blockage of bile ducts, or breakdown of a large number of red blood cells (this occurs in newborns).

2.3.2.2 Cholestasis

Cholestasis is reduced or blocked bile flow. This can either be intra-hepatic (occurring within the liver) or extra-hepatic (occurring outside the liver). Symptoms of cholestasis may include: jaundice, bone loss, easy bleeding, itching, small spider-like blood vessels visible in the skin, enlarged spleen, chills, fluid in the abdominal cavity and an enlarged gallbladder.

2.3.2.3 Hepatomegaly

Hepatomegaly (enlargement of the liver) is usually a positive indicator of liver disease. Usually, a slightly enlarged liver is not associated with any symptoms, while a grossly enlarged liver may present with symptoms of abdominal discomfort or "feeling full."

2.3.2.4 Portal Hypertension

This is an abnormally high blood pressure in the portal vein, due to increased pressure in the portal blood vessels or resistance to blood flow through the liver. Portal hypertension can lead to the growth of collateral vessels that will bypass the liver and connect directly to the general circulation, thereby conveying substances that are

normally removed by the liver into the general circulation. Symptoms may include a distended abdominal cavity and bleeding of the varicose veins at the lower end of the esophagus and in the stomach lining.

2.3.2.5 Ascites

Ascites is fluid build-up in the abdominal cavity, caused by fluid leaks from the surface of the liver and intestine. Ascites due to liver disease usually accompanies other liver disease indicators like portal hypertension. Ascites may present as distended abdominal cavity, which causes discomfort and shortness of breath.

2.3.2.6 Liver Encephalopathy

This is the deterioration of brain function resulting from the buildup of toxic substances which are normally removed by the liver. Liver encephalopathy is also called portal-systemic encephalopathy, hepatic encephalopathy, or hepatic coma. Symptoms typically comprise of impaired consciousness; changes in logical thinking, personality, and behavior; mood changes; impaired judgment; drowsiness; confusion; sluggish speech and movement; disorientation; loss of consciousness and coma.

2.3.2.7 Liver Failure

Liver failure, which is a severe deterioration of liver function, occurs when a large portion of the liver is damaged due to any type of liver disorder. Symptoms would normally include all the symptoms mentioned above in addition to general failing health, fatigue, weakness, nausea and loss of appetite.

2.3.2.8 Others

Symptoms specific to a particular type of liver disease can be seen in the autoimmune variant of chronic viral hepatitis, especially in young women, where manifestations may involve virtually any body system and can include acne, amenorrhea, arthralgia, ulcerative colitis, pulmonary fibrosis, thyroiditis, nephritis, and hemolytic anemia (Cohen, 2007). Chronic alcoholism in men causes signs of hypogonadism and feminization such as smooth skin, lack of male-pattern baldness, gynecomastia, testicular atrophy and changes in pubic hair. Under nutrition may lead to multiple vitamin deficiencies (e.g. folate and thiamin), enlarged parotid glands, and white nails. In alcoholics, Wernicke's encephalopathy and Korsakoff's psychosis result mainly from thiamin deficiency (Eldon, 2009c). Hepatocellular carcinoma may occasionally produce symptoms like hepatic friction rub or bruit and systemic metabolic complications (hypoglycemia, erythrocytosis, hypercalcemia, hyperlipidemia).

2.3.3 Diagnosis of liver disease

The available laboratory tests used assessing liver disorders have been found to be effective in detecting hepatic dysfunction; assessing the severity of liver injury; monitoring the course of the disease and its response to treatment. The most useful laboratory tests are the liver function tests which assess the biochemistry and excretory performance of the liver. The majority of liver function tests measure liver enzymes that are released into the bloodstream e.g. aminotransferases and alkaline phosphatase, while some measure the amount of protein in the blood. Only few of the tests actually assess liver function by evaluating hepatobiliary excretion e.g. bilirubin.

Specific diagnostic laboratory tests include (Eldon, 2009a):

- Immunoglobulin M (IgM) antibody to hepatitis A virus (anti-HAV) for acute hepatitis A
- Hepatitis B surface antigen (HBsAg) for hepatitis B
- Antibody to hepatitis C virus (anti-HCV) and HCV-RNA for hepatitis C
- Antimitochondrial antibody for primary biliary cirrhosis
- Serum ceruloplasmin (reduced) and urinary copper (elevated) for Wilson's disease
- α-Fetoprotein for hepatocellular carcinoma

2.3.3.1 Tests for Liver Injury

I. Aminotransferases: These are alanine aminotransferase (ALT), an enzyme found mainly in the liver and aspartate aminotransferase (AST), an enzyme found in the liver, heart, skeletal muscle, kidneys, and pancreas. Elevated ALT is to some extent specific for liver injury, whereas elevated AST may reflect rhabdomyolysis or injury to one of the organs in which it is found. Since these enzymes leak from damaged liver cells, they are sensitive indicators of liver injury. In most liver disorders, the ratio of AST to ALT is less than 1, while in alcoholic liver disease, the ratio is greater than 2 (Eldon, 2009a).

Markedly high values (> 500 IU/L; normal, ≤ 40 IU/L) indicate acute hepatocellular necrosis or injury. Modest elevations (300 to 500 IU/L) persist in chronic liver disorders (e.g. chronic hepatitis, alcoholic hepatitis) and in biliary obstruction, except where passage of a common duct stone can result in markedly high levels. Mild increases (< 300 IU/L) are nonspecific for a particular type of liver disorder and may present in conditions such as cirrhosis secondary to viral hepatitis, nonalcoholic fatty

liver disease, cholestatic liver disorders and hepatocellular cancer. Normal values can be seen in certain types of injuries e.g. hemochromatosis, drug induced liver injury and chronic hepatitis C (Eldon, 2009a).

II. Lactate dehydrogenase (LDH): LDH is present in the liver and many other tissues. It is an insensitive and nonspecific test for hepatocellular injury. Elevated levels are seen in ischemic hepatitis and cancers that extensively infiltrate the liver (Eldon, 2009a).

2.3.3.2 Tests for Cholestasis

I. Bilirubin: Bilirubin is a pigment in bile produced from the breakdown of heme proteins, mostly from the heme moiety of hemoglobin in old or damaged red blood cells. Elevated levels of bilirubin result in jaundice and dark urination. Three different tests of bilirubin are often used together:

- Total bilirubin - measures all the bilirubin in the blood. Normally, total bilirubin is mostly unconjugated, with values of < 1.2 mg/dL (< 20 μmol/L). Increased levels of unconjugated (indirect) bilirubin are seen in liver disorders such as Gilbert's syndrome.
- Direct bilirubin - measures conjugated bilirubin in the blood. Elevated levels result from decreased bile formation or excretion.
- Bilirubinuria - this reflects the presence of conjugated bilirubin in urine.

II. Alkaline phosphatase: This is a non-specific test because alkaline phosphatase consists of several isoenzymes and has a widespread extra-hepatic distribution (i.e. it is found in the placenta, small intestine, white blood cells, kidneys,

and bone). Increased levels of the enzyme suggest cholestasis, this occurs in disorders such as hepatitis, cirrhosis and syphilitic hepatitis. Levels of γ glutamyl transpeptidase or 5′-nucleotidase are more specific to the liver and are used to differentiate hepatic from extra-hepatic sources of alkaline phosphatase (Eldon, 2009a).

2.3.3.3 Tests for Synthetic Capacity of the Liver

I. Prothrombin Time (PT) and International Normalized Ratio (INR): PT or INR is a valuable measure of the liver's ability to synthesize fibrinogen and vitamin K–dependent clotting factors (factors II (prothrombin), V, VII, and X). An increasing PT or INR indicates severe hepatocellular dysfunction, a warning sign in acute liver disorders, while in chronic liver disorders; it signifies a progression to liver failure. These indices do not increase in mild hepatocellular dysfunction and are often normal in cirrhosis (Eldon, 2009a).

II. Serum proteins: Abnormalities in serum protein (albumin and globulin) are often non-specific for liver disorders. Serum globulin levels may be elevated in liver disease, rheumatoid arthritis and leukaemia, whereas serum albumin tends to decrease in liver dysfunction, dehydration, malnutrition and hypothyroidism.

2.3.3.4 Other Laboratory Tests

Other tests employed in the diagnosis of liver diseases, include:

I. Ammonia: Elevated levels occur in hepatic encephalopathy and advanced liver disorders.

II. Serum Immunoglobulins: Levels increase slightly in acute hepatitis, moderately in chronic active hepatitis, and markedly in autoimmune hepatitis. Different immunoglobulins are usually very high in different disorders e.g. IgM in primary biliary cirrhosis, IgA in alcoholic liver disease and IgG in autoimmune hepatitis.

III. Antimitochondrial antibodies: These heterogeneous antibodies are positive in patients with primary biliary cirrhosis, autoimmune hepatitis and drug-induced hepatitis.

IV. α-Fetoprotein (AFP): The slightest increase in AFP (normal ranges from < 10 to 20 mg/L) is often suggestive of primary hepatocellular carcinoma (HCC). Serum AFP generally correlates with tumor size, differentiation and metastatic involvement. Mild AFP elevations can also occur in acute and chronic hepatitis.

V. Specific hepatitis tests - To detect the different forms of viral hepatitis.

VI. Complete blood count (CBC) - To evaluate the patient's white and red blood cells and platelets. Normocytic anaemia, leucopenia and thrombocytopenia or pancytopenia are usually implicated in cirrhosis, while macrocytic anaemia, thrombocytopenia, and neutrophilic leukocytosis may be found in alcoholic liver disease.

VII. Tests for iron status - Accumulation of iron in the body leads to liver damage, a condition known as hemochromatosis.

VIII. Liver biopsy - This is usually carried out when the cause of liver disease is not known. The procedure involves inserting a small thin needle under the skin to obtain a tiny sample of liver tissue, which is analysed under a microscope to evaluate the integrity of the structure and cells of the liver.

2.3.3.5 Non-laboratory Tests

Medical imaging tests are examinations of still and moving images of internal organs and tissues. Imaging is essential for accurately diagnosing biliary tract disorders and detecting focal liver lesions such as abscess and tumors. It is limited in detecting and diagnosing diffuse hepatocellular disease such as hepatitis and cirrhosis.

Ultrasonography is the least expensive, safest, and most sensitive technique for imaging the biliary system. It differentiates intra-hepatic from extra-hepatic causes of jaundice and detects liver masses (Eldon, 2009a). CT scan (computed tomography) is commonly used to identify particularly small hepatic metastases, with an accuracy of about 80%. It is considered the most accurate imaging technique. However, the magnetic resonance imaging (MRI) is able to get more detailed images than a CT scan and is superior to both CT and ultrasonography for diagnosing diffuse liver disorders (e.g. fatty liver and hemochromatosis) and for clarifying some focal defects such as hemangiomas (Eldon, 2009a).

2.3.4 Prevention of liver diseases

Some liver diseases like viral hepatitis are infectious and can be prevented by adhering to the following (McLaughlin, 2000):

- practicing good hygiene, such as washing the hands after using the toilet or changing diapers
- avoiding drinking or using tap water when traveling internationally
- avoiding illegal drug use, especially sharing injection equipment
- practicing safe sex
- avoiding the sharing of personal hygiene items, such as razors or nail clippers
- avoiding toxic substances and excess alcohol consumption
- using medications only as directed
- eating a well balanced diet
- getting vaccinated against hepatitis A and B
- using officially recommended safety precautions in healthcare and day care work

2.3.5 Treatment of liver diseases

The treatment for liver diseases is usually aimed at relief of symptoms and complications. Generally, treatment may comprise of bed rest, rehydration, adequate nutrition and anti-nausea medications when required. Further treatment is dependent on the type and severity of the disease. Specific pharmalogical interventions include:

- Wilson's disease is treated with penicillamine at a dose of 0.75-1.5g per day; or zinc acetate at 50 mg (for patients weighing 50kg and above) or 25 mg (for patients aged 5-18 weighing less than 50kg) three times daily.
- Hemochromatosis is treated by removing a pint of blood once or twice a week for several months to a year to reduce the excess iron.

- Patients with biliary atresia undergo a procedure called the Kasai surgery, in which the the bile ducts are replaced with part of the intestine (McLaughlin, 2000).
- For patients with cholestatic hepatitis, 8 g of cholestyramine once or twice a day can be administered orally to relieve itching.
- A corticosteroid with or without azathioprine is usually prescribed for autoimmune hepatitis. Corticosteroids such as prednisone are administered orally at a dose of 30 to 40 mg daily, and then tapered to the lowest dose that maintains aminotransferases at normal or near-normal levels. Azathioprine is administered either concomitantly or when prednisone fails to maintain suppression, at an oral dose of 1 to 1.5 mg/kg daily (Cohen, 2007).
- Antiviral treatment is indicated for HBV-infected patients with elevated aminotransferase levels, clinical or biopsy evidence of progressive disease, or both. Treatment is usually expensive as it may need to be continued indefinitely. Stopping treatment prematurely can lead to relapse, which may be severe. Six antiviral drugs are commonly used in such infections, they are:
    - ✓ Entecavir, an oral nucleoside analogue often used as a first-line drug at a dose of 0.5 mg daily. It has a higher antiviral potency than other commonly used drugs and resistance to the drug is uncommon.
    - ✓ Adefovir, an oral nucleotide analogue may be given in combination with entecavir or alone as a first line drug at a dose of 10 mg daily. It may cause renal dysfunction.
    - ✓ Lamivudine, a nucleoside analogue given at 100 mg orally/day. It has fewer adverse effects compared to other antivirals used to treat HBV infection.

- ✓ Interferon α (INF-α), given at a dose of 5 million IU subcutaneously daily or 10 million IU subcutaneously 3 times a week for 4 months.
- ✓ Pegylated INF-α2a (peginterferon-α2a), given at a dose of 180 μg subcutaneously once a week.
- ✓ Telbivudine is a new drug that has greater efficacy than lamivudine but has high rates of resistance.

- For chronic hepatitis due to HCV, treatment is indicated if aminotransferase levels are elevated and biopsy shows active inflammatory disease with evolving fibrosis. Combination therapy with pegylated IFN-α (IFN-α2b at 1.5 μg/kg or IFN-α2a 180 μg) subcutaneously once a week plus ribavirin (400 to 600 mg) orally twice a day, gives the best results.
- Abstinence or restriction of alcohol intake can be achieved by administering drugs such as baclofen and disulfiram. Baclofen has a short-term benefit of reducing the craving and withdrawal symptoms, while disulfiram causes unpleasant side effects in the presence of alcohol. Benzodiazepines may be used to calm or sedate patients experiencing alcohol withdrawal symptoms (not for patients with advanced alcoholic liver disease as it can precipitate alcoholic encephalopathy). For severe alcoholic hepatitis corticosteroids (prednisolone 40mg/day, orally for 4 weeks, followed by tapered doses) can be administered.
- Preliminary evidence suggests that thiazolidinediones can help correct biochemical and histologic abnormalities in NASH (Herrine, 2009).

Liver transplantation is often the best option for either liver cancer or cirrhosis. Most end-stage liver disease patients are unfortunately faced with the limitations of severe

shortage in the number of donor organs available and strict restrictions on patient eligibility for liver transplants.

2.3.6 Role of medicinal plants in the treatment of liver diseases

The inadequacy of orthodox medicines in the management of liver diseases is apparent in the continuing premature deaths and disabilities resulting from these diseases. Traditional medicine has over time offered, in the form of medicinal plants, an alternative to this malady. Quite a number of plants are used in different parts of the world for the treatment of liver diseases, such as; leaves of *Berberis lycium* (Chopra *et al.*, 1986); an extraction of the leaves of *Cynodon dactylon* and *Phyllanthus amarus* grounded with the fruits of *Piper nigrum*; the powdered root of *Hemidesmus indicus* mixed with honey; fried leaves of *Azadirachta indica* and *Carum nothum*, salted, powdered and taken with milk (Shanmugam, 2009).

Others are the interior stem portion of *Musa paradisiaca* taken with honey or mixed with the fruits of *Lablab purpureus* and taken as a vegetable curry; powdered *Aegle marmelos* leaf mixed with Goat milk; and the fried, dried, fruit pieces of *Momordica charantia* among others. From the inexhaustible list of locally used remedies for the treatment of liver diseases, a mixture of three plants used as a local remedy in Zaria has been selected for this research; they are *Boswellia dalzielii*, *Balanites aegyptiaca* and *Ipomoea asarifolia*.

2.3.6.1 Balanites aegyptiaca (L.) Delile

- Scientific name: *Balanites aegyptiaca*
- Common names (Burkill, 1985):

English: Egyptian balsam, Soapberry tree, Thorn tree

Nigeria: Fulfulde- Morotodi, Tanni

Hausa- Aduwa, Balangade

Kanuri- cíngó

- Taxonomic Hierarchy (ITIS report, 2009):

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Sapindales

Family: Zygophyllaceae

Genus: Balanites Del.

Species: Balanites aegyptiaca (L.) Del.

- Description:

The Balanites are plants of the Zygophyllaceae family, comprising 9 species and 11 intraspecific taxa. *Balanites aegyptiaca* which is one of the most common species of the genus Balanites is a widely grown desert tree with a multitude of potential uses. It is a small evergreen thorny tree found throughout the Sudano-Sahelian region of Africa and in other arid and semi arid regions of Africa, the Middle East, India and Burma. Various parts of the Balanites tree are used for folk medicines in many regions of Africa and Asia (Neuwinger, 1996; Mohamed *et al*., 2000; Mohamed *et al*., 2002).

The tree can grow to 6-10 meters in height, is highly resistant to stresses such as sandstorms and heat waves, grows with minimal available moisture (drought-resistant)

and can live and remain productive for dozens of years. The tree produces date-like fruits, has thick, tough glossy leaves, spiny branches, and a double root system. Flowering period is between the months of February and August, during which the tree produces yellow or green flowers with five petals. The plants grow extensively even when neglected. One estimate is that more than 400,000 tons of Balanites fruit are produced in Sudan alone (Mohamed *et al.*, 2000). It can successfully grow in a marginal sand dune with saline and sewage water.

Phytochemical investigations on *Balanites aegyptiaca* resulted in the isolation of several classes of secondary metabolites, many of which expressed biological activities such as coumarins, flavonoids and steroidal saponins (Sarker *et al.*, 2000). Several steroidal saponins and yamogenin glycosides have been isolated from the roots and bark of the tree (Liu and Nakanishi 1982; Petit *et al.*, 1991). Two furostanol glycosides and 6-methyl-diosgenin were also obtained from the fruits (Hosny *et al.*, 1992; Kamel 1998). More recently five new steroidal glycosides were isolated from the roots of the plant (Farid *et al.*, 2002).

- Important Uses:

I. Ethnomedicinal:

Plant tissues from *B. aegyptiaca* have been used in a variety of folk medicines in Africa and Asia. Extracts from several parts of this tree have been intensively used in Africa and India for various ethnobotanical purposes. For example, Balanites extracts have been shown to exhibit antifeedant, antidiabetic, molluscicide, antihelmintic and contraceptive activities (Iwu, 1991; Kamel *et al.*., 1991; Liu and Nakanishi, 1982; Ibrahim, 1992; Rao *et al.*., 1997). The fruit is also known to kill the snails which carry

schistosomiasis and bilharzia flukes (Tredgold, 1986). Several studies have reported that the presence of saponins (amphiphilic molecules consisting of a hydrophobic sterolic aglycone linked to one or more hydrophilic glycoside chains) in *B. aegyptiaca* is the main cause behind these activities (Pettit *et al.*., 1991; Hosny *et al.*., 1992; Kamel, 1998).

The fruits have been used in the treatment of liver and spleen diseases. The bark, unripe fruits, and leaves of this plant are reported to have antihelmintic, antifertility, purgative and anti-dysentric properties (The Useful Plants of India, 1994). An aqueous suspension of dried fruits of this plant is being used as an abortifacient by local herbal healers. The roots are used for abdominal pains and as a purgative. In Zaria, Nigeria, the roots are used together with other plants for treatment of jaundice. The Gum from the wood is mixed with maize meal porridge to treat chest complaints (FAO, 1993).

II. Food:

The fruit of *B. aegyptiaca* consists of an epicarp (5-9%), a mesocarp (28-33%), an endocarp (49-54%) and an oil-rich kernel (8-12%) which is used by the local people as a source of edible oil (Zeev, 2008). The fruit pulp though bitter, is edible. It produces fruit even in dry years which makes it a highly appreciated food source in dry areas. Pounded fruits make a refreshing drink which becomes alcoholic if left to ferment.

III. Aesthetics:

In the past, Egypt was the centre of the production of great scents. The base oil for some of the important scents was derived from the kernel of the *Balanites aegyptiaca*. The perfumers also used the bruised husks of the fruit for its fragrance.

IV. Others:

B. aegyptiaca has fine-grained dense and heavy heartwood; it is easily worked and takes a good polish. Although valued for furniture it may be twisted and difficult to saw. The wood is durable and resistant to insects making it good for tool handles and domestic items such as spoons (FAO, 1993). Root cuttings readily form a live fence. Protein rich leaves and shoots are an excellent source of fodder. The leaves make very good mulch. The tree is also valued as firewood since it produces almost no smoke and has a calorific value of 4600 kcal per kg (Webb *et al.*, 1984).

Although *B. aegyptiaca* has been used for many purposes—from ethnobotanicals to firewood, from forage to edible fruit, this plant is considered one of the most neglected species of arid regions and has yet to be domesticated.

- Research Activities on *B. aegyptiaca*:

Mohamed *et al.* (1999) reported that the aqueous extract of *Balanites aegyptiaca* was found to have no effect on rabbit intestine, rabbit aortic strip, rat stomach strip, rat uterus and rat phrenic nerve-diaphragm in a dose up to 10 mg/mL gut bath. At a higher dose (25 mg), the extract decreased significantly the contractility and the rate of the isolated perfused rabbit heart. Administration of the aqueous extract to biliary duct-ligated rats, showed a dose-dependent significant decrease in serum bilirubin level in experimental obstructive jaundice in rats.

In another study, Ali *et al.* (2001) examined the effect of the lyophilized extract of *Balanites aegyptiaca* bark on liver damage induced by paracetamol in mice. Their results showed that oral pretreatment of mice with the extract protected only 27% of the

animals against the lethal effect of paracetamol (1 g/kg), showing a relatively modest hepatoprotective activity.

A method for the production of biodiesel from *Balanites aegyptiaca* oil or crushed nuts was invented by Mordechay *et al*. in 2008. They reported that the biodiesel obtained had a composition of triglycerides of mainly C16:0 and C18:0 saturated and unsaturated fatty acids, with a very high content of linoleic acid and oleic acid, it was also found to contain balanites saponins, acting as surfactants, which reduced the rate of corrosion and improved the performance of the engine.

Ethanolic and petroleum ether extracts of *Balanites aegyptiaca*, have been found to dose dependently reduce carrageenan-induced paw edema in rats and increase reaction time in both Eddy's hot plate method and tail-flick method in albino mice, with the ethanolic exhibiting a higher activity than petroleum ether (Gaur *et al*., 2008).

Using the Trolox assay, the fruit and seeds of the plant were observed to possess strong antioxidant activity (Cook *et al.*, in 1998), in addition to being effective in the management of HIV/Aids and Leukemia (Hamid *et al.*, 2001).

2.3.6.2 Boswellia dalzielii Hutch

- Scientific name: *Boswellia dalziellii*
- Common names (Burkill, 1985):

English: Frankincense tree

Nigeria: Fulfulde- Juguhi, anndakehi, janawhi, soma

Hausa- Ararraßi, ba-samu, hánoó, hánuú, Chibđi

Kanuri- Káfî dukkán

- Taxonomic Hierachy:

Family: Burseraceae

Genus: Boswellia

Specific epithet: Dalzielli

- Description

The genus Boswellia consists of few species widespread throughout the world. *Boswellia dalzielli* is a tree that grows up to 13 m in height, with slender ascending branches and a characteristically smooth, pale brown bark that peels off in ragged papery patches. It is found in savanna regions, locally abundant in Northern Ivory Coast, Northern Nigeria, Cameroun and Ubangi-Shari. The small white flowers which may appear while the tree is leafless are fragrant. The tree is sometimes planted in northern parts of Ivory Coast and Upper Volta as an ornamental tree (Kerharo & Bouquet, 1950).

The bark contains a fragrant whitish exudate which dries readily and is friable. The exudate is an oleo-gum-resin; the gum contains bassorin and the resin boswellinic acids. The aqueous stem bark extract of *Boswellia Dalzeilii* contains various pharmacologically active compounds, such as, alkaloids, tannins, saponins and anthraquinones (Etuk *et al.*, 2006). Kubmarawa *et al.* (2006) reported that the complex essential oil found in the leaves contains hydrocarbons, alcohols, esters, carbonyls, oxides and epoxides, of which the most abundant constituents were α-pinene (45.7%) and α-terpinene (11.5%). Other compounds that could be identified in significant proportions included trans-sabinene hydrate (4.6%), cis-p-menth-2-en1-ol (2.9%), α-campholenal (2.7%), caryophyllene oxide and α-phellandrene (2.3%). They also added

that the abundance of monoterpenoid compounds (84.3%) in the oil of the studied *B. dalzielli* is in accordance with previous findings for other species in this genus.

- Important Uses

I. Ethnomedicinal:

Boswellia dalzielii is very popular among the locals as a potent source of ethnomedicine (Nwude and Ibrahim, 1980). A bark-decoction is used as an antiseptic wash for sores in Ivory Coast, and is an ingredient of a complicated prescription for leprosy (Bouquet & Debray, 1974; Kerharo & Bouquet, 1950). In Northern Nigeria the bark is boiled up in large quantity to make a wash for fever, rheumatism, etc., and the fluid is taken internally for gastro-intestinal troubles (Dalziel, 1937). The Fulani of Northern Nigeria use a cold infusion of the bark for snake-bite. The fresh bark is eaten in Adamawa to cause vomiting after a few hours thus relieving symptoms of giddiness and palpitations (Dalziel, 1937).

The root and bark are held to be antidotes to arrow-poison, e.g., the root is combined with that of *Daniellia oliveri* (Rolfe) to make a decoction which is drunk by the wounded person and is said to be effective without causing diarrhoea (Dalziel, 1937). A root-decoction boiled with *Hibiscus sabdariffa* (Linn) is taken in copious draughts as a remedy for syphilis (Dalziel, 1937). The gum has been used in western medicine in fumigatory preparations, and sometimes in plasters and in urinary antiseptics, while the resin is used as a diuretic and a remedy for kidney diseases. The extract from the leaves of the plant is used for the treatment of diarrhea in poultry (Dalziel, 1937).

II. Others

It is planted as a village stockade in Northern Nigeria and often as a live-fence to bring prosperity (ba-samu) or to prevent (hano) bad luck, hence the Hausa names. The exudates from the bark is burned alone or with other fragrant resins to fumigate clothing and in rooms to drive out flies, mosquitoes, etc. (Dalziel, 1937). It is used by Catholic Missions as a substitute for true incense (Kerharo & Bouquet, 1950). It may be added to the juice of Acacia berries used in ritual mummifications practiced by various tribes in Northern Nigeria (Meek, 1931). Along with an extract of the bark of *Vitex doniana* Sweet (Verbenaceae), it is sometimes an ingredient of malam's ink (Dalziel, 1937).

- Research Activities on *B. dalzielii*

Etuk *et al.* (2006) reported that the aqueous stem bark extract of *Boswellia dalzielii* could be considered relatively safe from acute toxicity especially when given orally and that prolonged oral administration of very high doses of the aqueous stem bark extract of *Boswellia dalzielii* may be associated with increased risk of toxicity (Di Josephg *et al.*, 1984). Liver function screening revealed that the plant had no significant effect on total protein and albumin content, but showed a dose dependant decrease in unconjugated bilirubin and transaminase levels (Aliyu *et al.*, 2007). Crude extracts of its stem bark have been found to show antibacterial activity against gram-positive and gram-negative bacteria (Olukemi *et al.*, 2005). The aqueous stem bark was reported to show anti-ulcer activity and reduced gastrointestinal motility; also oil from the leaves was found to exhibit significant activity against S. aureus, B. subtilis and C. albicans (Nwinyi *et al.*, 2004).

2.3.6.3 Ipomoea asarifolia (Desr.) Roem. & Schult.

- Scientific name: *Ipomoea asarifolia*
- Common names:

English: Ginger-leaf morning glory

Nigeria (Burkill, 1985):

Fulfulde- layre ngabbu

Hausa- dúmán kada, dúmán raàfií

Yoruba:- gbọọrọ ayaba

- Taxonomic Hierarchy (ITIS report, 2009):

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Solanales

Family: Convolvulaceae

Genus: Ipomoea L.

Species: *Ipomoea asarifolia* (Desr.) Roemer & J.A. Schultes

- Description:

Ipomoea asarifolia belongs to the convolvulaceae family which comprises about 58 genera and 1650 species, widely distributed in tropical, subtropical and temperate regions. It is a long-trailing herbaceous perennial, sometimes twining, with hollow stems and large red-purple flowers. It is found in sandy areas and waste places throughout Africa, and in Cape Verde Islands, tropical Asia and America. In Africa, it

is predominantly found in Nigeria, Sudan, Senegal, Mali, Angola, Mozambique, Ghana, Ivory Coast and Cameroon.

The plant is attractive to bees, butterflies and birds. It has very high moisture needs; suitable for bogs and water gardens. It is neither eaten by man nor normally by livestock and is believed to contain a purgative toxic resin. In Northern Nigeria, it is said to cause diarrhoea in horses if accidentally grazed, and madness and death in camels (Dalziel, 1937). The plant is however used for medicinal purposes, particularly in Africa.

Ergoline alkaloids as well as volatile oils have been found to be present in *Ipomoea asarifolia*. The leaf contains a crude protein level of 32% and a good mineral profile. Two triacylated and tetraglucosylated anthocyanins derived from cyanidin have been isolated from the flowers of *Ipomoea asarifolia* (Kouda-Bonafos, 2003).

- Important uses:

I. Ethnomedicinal:

Ipomoea asarifolia is used in Senegal for various gynaecological purposes: urinary problems during pregnancy, haemorrhage, as an ecbolic and abortifacient; also in general as a wound-dressing, and for treating ophthalmias, neuralgia, headaches, arthritic pain and stomach-ache (Kerharo and Adam, 1963; Kerharo and Adam, 1964; Kerharo and Adam, 1974). The pulped-up leafy stems are mixed with citron and water and taken in Ivory Coast as an ecbolic (Kerharo & Bouquet, 1950).

In Northern Nigeria, a leaf-decoction is taken internally and as a wash for feverish chills and rheumatic pains, or the face is steamed over a hot decoction of the plant along with husks of bulrush millet (Dalziel, 1937). A leaf-poultice is applied to guinea-worm sores in and the flowers boiled with beans are eaten as a remedy for syphilis in Nigeria (Dalziel, 1937). In the Northern Territory of Ghana cattle affected with garli disease are treated by concoctions of stems and roots of various plants of which this is usually the most important ingredient. An infusion of the materials is given to the animal internally, and a charcoal of the burnt plants is pulverized, mixed with Shea butter and rubbed on the joints (Dalziel, 1937).

II. Agriculture:

The plant trailing over sand-dunes is a good sand binder (Irvine, 1930). The long stems are used as ties for tying up produce (Dalziel, 1937). In Senegal certain agriculturists growing groundnuts stretch out the long runners over the top end of stakes in their fields as ‘a good example’ to the groundnuts to encourage the plants to emulate the long stems and many pods of the Ipomoea (Trochain, 1940). In Nigeria, the leaf of *Ipomoea asarifolia* has no known food value and thus popularly used as compost material, mulch, as well as constituting weed in farms.

III. Others:

A decoction of the plant is used to stain cloths and the hair black, while its ashes are mixed with indigo to provide a blue dye for cloth or ashes of the leaves alone are used. The dried stems are used as tinder, and the leaves are sometimes used to wrap the feet or hands in applying henna (Dalziel, 1937).

- Research Activities on *I. asarifolia:*

In a study conducted by Medeiros *et al.* (2003), it was observed that goats administered greater than 5 g/kg plant showed clinical signs characteristic of tremorgenic syndrome (mild depression, slight tremors of the muscles of the head, neck and head nodding with signs more apparent after exercise).

So also, Araujo *et al.* (2008) reported that the fresh plant was found to be more toxic during the dry season than the rainy season, it maintains its toxicity even after being dried and that sheep are more susceptible to the poisoning than goats. They also reported that pregnant sheep alone showed signs of toxicity and not their lambs, suggesting that the active principle is not eliminated through the milk at doses toxic for the lambs. It was however found that *Ipomoea asarifolia* leaf meal (IALM) could be included in weaner pig diets up to a 15% level without deleterious effect but optimally at a 10% level (Ekenyem, 2006).

Pharmacologically, Jegede *et al.* (2009) reported that 70% methanolic extract of the leaves of *Ipomoea asarifolia* at doses of 100-400 mg/kg i.p. significantly ($P<0.05$) reduced pain and inflammation at the late phase of the process. The stem bark, leaves and root bark of the plant have also been shown to possess significant antioxidant properties (Atawodi and Onaolapo, 2010).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Collection of Plant Materials

Balanites aegyptiaca root, *Boswellia dalzielii* stem bark and *Ipomoea asarifolia* leaves were collected from the woodlands of Zaria during the rainy season in June, 2009. Bulk collection was carried out and plant samples were identified at the herbarium of the Department of Biological Sciences, Ahmadu Bello University (ABU) Zaria, where voucher numbers were assigned them (*B. aegyptiaca*- 2064; *B. dalzielii*- 2448; *I. asarifolia*- 1060). The plants were authenticated at the Department of Medicinal Plant Research, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja.

3.2 Extraction

Balanites aegyptiaca root, *Boswellia dalzielii* stem bark and *Ipomoea asarifolia* leaves were cleaned, air dried, powdered separately, and individually subjected to cold maceration in 70% methanol for 48 hours, after which the mixtures were filtered with a clean muslin cloth and the resultant marc discarded. Each filtrate was placed on a thermostat water bath at 100˚C to evaporate the solvent. The resulting extracts were allowed to cool, weighed and stored in the fridge. Solutions of the extracts were freshly prepared for each study.

3.3 Animals

Wistar albino rats of either sex, weighing between 120-200g, were bought from the animal house of the Department of Pharmacology, ABU Zaria. The animals were transported to the animal facility at NIPRD, where they were allowed to acclimatize for

two weeks before commencing the study. They were maintained under standard laboratory conditions of temperature (22±1˚C), relative humidity (44±1%) and 12-hour light, 12-hour dark cycles. The rats were fed with standard laboratory animal feed and given water *ad libitum* throughout the period of the study. All experiments were performed according to the National Institutes for Health principles of laboratory animal care (NIH Publication No. 85; rev. 1985).

3.4 Chemicals and Drugs

Methanolic extracts from each of the three plants i.e. *Balanites aegyptiaca, Boswellia dalzielii, Ipomoea asarifolia* and their mixture were used as the test drugs. Silymarin (MADAUS Gmbh, Germany), 99.9% carbon tetrachloride (Sigma Chemical Co. USA), 70% methanol (Sigma Chemical Co. USA) and Pentobarbitone sodium (Sigma Chemical Co. USA) were used.

3.5 LD_{50} and Acute Oral Toxicity Studies in Rats

Acute oral toxicity studies were performed using the Acute Toxic Class Method of the OECD guideline for testing of chemicals (OECD-423, 2001). The method is based on biometric evaluations of fixed doses, adequately separated to enable proper classification and hazard assessment of substances (Diener and Schlede, 1999). The original guideline adopted in1996, was extensively validated *in vivo* against LD_{50} data obtained from literature (Schlede *et al.*, 1992; Schlede *et al.*, 1994), allowing the acute toxic class method to be used as an alternative to the median lethal dose (LD_{50}) tests.

Four groups, each comprising three female rats were used for the study. The animals were fasted overnight (from 6pm-8am) provided only with water, after which the three

methanolic extracts and their mixture i.e. one extract per group, were administered to the animals by gastric intubation, at a dose of 300 mg/kg body weight. The appearance and disappearance of toxic symptoms such as behavioral changes, locomotion, convulsions and mortality, were observed and recorded. The animals were observed constantly for the first 30 minutes after administration and periodically for the rest of the day, with special attention given during the first 4 hours. Thereafter, they were observed daily for a total of 14 days.

Since no mortality was observed at 300 mg/kg, another set of twelve female rats, divided into four groups of three animals each, were given oral administrations of the extracts and their mixture at a dose of 2,000 mg/kg body weight (one extract per group). The animals were observed (as mentioned above), appearance and disappearance of toxic symptoms were noted and the LD_{50} cut-off was determined from the test chart. One-tenth of the LD_{50} was selected as the therapeutic dose for the evaluation (Jalalpure *et al.*, 2003). Two additional doses were also selected for the study as follows:

1^{st} dose = therapeutic dose = 0.1 x LD_{50}

2^{nd} dose = therapeutic dose x 2

3^{rd} dose = $\frac{\text{therapeutic dose}}{2}$

3.6 Drug Preparations

Solutions of the plant extracts were obtained by dissolving appropriate quantities of the extracts in distilled water to obtain stock solutions of 400 mg/ml, 200 mg/ml and 100 mg/ml for each extract. To obtain the mixture, the median effective dose (ED_{50}) for each extract was determined from its dose-response curve and the quantities were

mixed together and dissolved in distilled water. The resulting stock solution contained 160 mg/ml of *Balanites aegyptiaca,* 220 mg/ml of *Boswellia dalzielii* and 300 mg/ml of *Ipomoea asarifolia.*

3.7 Phytochemical Screening

Phytochemical screening for the extracts was carried out using standard screening procedures adopted from Wall *et al.* (1952 and 1954). Each extract was screened for the presence of alkaloids, flavonoids, tannins, terpenes, glycosides, sterols, saponins, resins, phenols and volatile oils, using the methods described below:

3.7.1 Test for alkaloids

0.5 g of each extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath. 1 ml of the resulting filtrate was treated with a few drops of mayer's reagent and a second 1 ml portion was treated with a few drops of dragendorffs reagent. Turbidity or precipitation with either of the reagents signified the presence of alkaloids (Harbone, 1973).

3.7.2 Test for saponins

0.5 g of each extract was shaken with 2 ml of distilled water in a test tube. Frothing, which persisted on warming was taken as an evidence for the presence of saponins (Wall *et al.,* 1952 and 1954).

3.7.3 Test for tannins

1 g of each extract was stirred in 2 ml of distilled water and then filtered. A few drops of ferric chloride reagent were added to the filtrate. The appearance of a blue-black,

green or blue-green precipitate showed the presence of tannins in the extracts (Trease and Evans, 2002).

3.7.4 Test for glycosides

10 ml of 50% sulphuric acid was added to 1 ml of each plant extract in a test tube and the mixture heated in boiling water for 15 minutes. 2 ml of Fehling's solution was added and the mixture boiled. A brick-red precipitate indicated the presence of glycosides (Okerulu and Ani, 2001).

3.7.5 Test for flavonoids

1 g of each extract was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered and divided into two portions. 5 ml of 10% sodium hydroxide was added to 5 ml of the first portion of the detanned extract. A yellow solution indicated the presence of flavonoids (Sofowora, 1993).

3.7.6 Test for phenols

2 ml of the detanned water extract above was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A green or blue solution indicated the presence of a phenolic nucleus.

3.7.7 Test for terpenes

5 g of each extract was macerated in 50 ml of 95% ethyl acetate, filtered, and the filtrate evaporated to dryness. The residue was dissolved in 10 ml of anhydrous chloroform and then filtered. The filtrate was divided into two equal portions. The first

portion was mixed with 1 ml of acetic anhydride, and then 1 ml of concentrated sulphuric acid was poured down the wall of the test tube to form a layer underneath. The formation of a reddish violet colour signified the presence of terpenes (Sofowora, 1993).

3.7.8 Test for sterols

To the second portion of the chloroform solution above, 2 ml of concentrated sulphuric acid was added carefully, such that the acid formed a layer beneath the chloroform solution. A reddish brown colour indicated the presence of a steroidal ring (Sofowora, 1993).

3.7.9 Test for resins

0.1 g of each extract was dissolved in 15 ml of petroleum ether and filtered into a test tube. 15 ml of copper acetate solution was added and shaken vigorously, then allowed to separate. Appearance of a green colour showed the presence of resins.

3.7.10 Test for volatile oils

Small quantities of each extract were shaken with 0.1M sodium hydroxide and dilute hydrochloric acid. A white precipitate was formed in the presence of volatile oils (Brain and Turner, 1975).

3.8 Hepatoprotective Studies

The prophylactic and curative properties of the extracts were assessed using two methods:

3.8.1 Direct method

The method described by Reddy *et al.* (1992) with slight modifications, was used for the experiment. Modifications were based on preliminary studies, which revealed that intravenous administration of the toxin on alternate days would be more effective for optimal induction of hepatotoxicity. The extracts on the other hand were administered daily.

3.8.1.1 Prophylactic Studies

For the prophylactic study, the rats were divided into seven groups of six rats each and treated as follows:

I. Group I- received only the vehicle, distilled water at a dose of 1 ml/kg/day p.o. for twelve (12) days.

II. Group II- received the vehicle, distilled water at a dose of 1 ml/kg/day p.o. for the first seven days, followed by 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) i.p for the next five days.

III. Group III- received the standard drug, silymarin at a dose of 100 mg/kg/day p.o. for the first seven days, followed by 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) i.p for the next five days.

IV. Groups IV to VI- each were made up of three sub-groups of six animals each, to represent individual dose levels for each of the three extracts (3 doses per extract i.e. 100mg/kg, 200mg/kg and 400mg/kg). Each sub-group received a different dose of the extract p.o. for the first seven days, and then 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) i.p for the next five days.

V. Group VII- received oral administrations of 160mg/kg *B. aegyptiaca*, 220mg/kg *B.dalzielii* and 300mg/kg *I. asarifolia* mixed together as a preparation for the first seven days, then 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) i.p for the next five days.

3.8.1.2 Curative Studies

The rats were divided into seven groups of six rats each and treated as follows:

I. Group I- received only the vehicle, distilled water at a dose of 1 ml/kg/day p.o. for twelve (12) days.

II. Group II- received i.p injections of 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) for the first five days, followed by the vehicle, distilled water at a dose of 1 ml/kg/day, p.o. for next seven days.

III. Group III- received i.p injections of 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) for the first five days, then the standard drug, silymarin at a dose of 100 mg/kg/day p.o. for the next seven days.

IV. Groups IV to VI- each were made up of three sub-groups of six animals each, to represent individual dose levels for each of the three extracts (3 doses per extract i.e. 100mg/kg, 200mg/kg and 400mg/kg). All the groups received i.p injections of 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) for the first five days, then each sub-group was administered a different dose of the extract p.o. for the next seven days.

V. Group VII- received i.p injections of 0.5 ml/kg of 99.9% carbon tetrachloride mixed with olive oil (1:1) for the first five days, followed by oral administrations of 160mg/kg *B. aegyptiaca*, 220mg/kg *B.dalzielii* and

300mg/kg *I. asarifolia* mixed together as a mixture for the first next seven days.

3.8.2 Indirect method

This method employed the barbiturate-induced sleep model described by Anand *et al.* (1992) to assess both prophylactic and curative properties of the extract. The experiment was set up as described above for both curative and prophylactic studies, both of which lasted for 12 twelve days, on the 13th day of the experiments, all the animals were administered with 25 mg/kg i.p of pentobarbitone sodium and observed for sleep (loss of righting reflex). The interval between the time pentobarbitone was administered and the time righting reflex was lost, was recorded as the onset of sleep, while the time interval between loss and regain of righting reflex was recorded as Pentobarbitone Sleeping Time (PST) for each animal.

3.9 Biochemical Parameters

The animals were euthanized on the 13th day and blood samples were collected separately by cardiac puncture into lithium heparin bottles and sent for analysis. Standard operating procedures (Afia and Momoh, 2006) were used to conduct assays for the following biochemical parameters:

3.9.1 Alkaline phosphatase - ALP (KINETIC Reaction)

Reagents: Diethanolamine buffer 1.25 mol/L pH 10.2

Magnesium chloride 0.6 mmol/L

4-nitrophenyl phospate 50 mmol/L

Biocides

Sample: Serum

Cuvette: 1 cm light path

Wavelength (λ): 405 nm

20 µl of the sample and 1 ml of the reagent were pipette into a cuvette and mixed gently by inversion. The cuvette was placed into the cell holder of a photometre and incubated for 1 min. The initial absorbance reading was recorded and readings were repeated exactly after 1, 2, and 3 min. The difference between absorbances was calculated and the mean of the results computed to obtain the average change in absorbance per min (△ A/min).

Calculation for ALP: µL = △ A/min x 2764

3.9.2 Aspartate transaminase - AST

Reagent 1: Phosphate buffer 100 mmol/L pH 7.4

L-aspartate 100 mmol/L

α-oxoglutarate 2 mmol/L

Reagent 2: 2,4-dinitrophenylhydrazine 2 mmol/L

Sample: Heparinised plasma and serum

Cuvette: 1 cm light path

Wavelength (λ): 546 (530-550) nm

Two test tubes, one for the reagent blank and the other for the sample were used for the assay. 0.5 ml of reagent 1 and 0.1 ml of distilled water were pipette into the reagent blank test tube. In the sample test tube, 0.1 ml of the sample and 0.5 ml of reagent 1 were added. Both test tubes were mixed gently and incubated for 30 min at 37^{o}C, after which 0.5 ml of reagent 2 was mixed into the two tubes and allowed to stand for exactly 20 min at 20-25^{o}C. 5 ml of 0.4M sodium hydroxide was then added into the

two test tubes and mixed. Absorbance of the sample was read against that of the reagent blank after 5 min. AST activity was obtained from the serum/plasma table provided.

3.9.3 Alanine transaminase – ALT

Reagent 1:	Phosphate buffer 100 mmol/L pH 7.4
	L-alanine 200 mmol/L
	α-oxoglutarate 200 nmol/L
Reagent 2:	2,4-dinitrophenylhydrazine 2 mmol/L
Sample:	Serum and plasma
Cuvette:	1 cm light path
Wavelength (λ):	546 (530-550) nm

0.5 ml of reagent 1 and 0.1 ml of distilled water were pipette into the reagent blank test tube. In the sample test tube, 0.1 ml of the sample and 0.5 ml of reagent 1 were added. Both test tubes were mixed gently and incubated for 30 min at 37^{o}C, after which 0.5 ml of reagent 2 was mixed into the two tubes and allowed to stand for 20 min at 20-25^{o}C. 5 ml of 0.4M sodium hydroxide was then added into the two test tubes and mixed. Absorbance of the sample was read against that of the reagent blank after 5 min. ALT activity was obtained from the serum/plasma table provided.

3.9.4 Bilirubin (colorimetric - randox method)

Reagent 1:	Sulphanilic Acid 29 mmol/L
	Hydrochloric acid 0.17N
Reagent 2:	Sodium Nitrite 25 mmol/L
Reagent 3:	Caffeine 0.26 mol/L
	Sodium benzoate 0.52 mol/L

Reagent 4:	Tartrate 0.93 mol/L		
	Sodium hydroxide 1.9N		
Sample:	Serum		
Cuvette:	1 cm light path		
Temperature:	20-25^{o}C		
Wavelength (λ):	Total bilirubin	-	578 (560-600) nm
	Direct (conjugated)	-	546 (530-560) nm

3.9.4.1 Total bilirubin (TBIL)

0.2 ml of reagent 1, 1 ml of reagent 3 and 0.2 ml of the sample were mixed together in a test tube (sample blank) and allowed to stand for 10 min. In another test tube (sample), 0.2 ml of reagent 1, 1 drop of reagent 2, 1 ml of reagent 3 and 0.2 ml of the sample were mixed together and also allowed to stand for 10 min. 1 ml of reagent 4 was then added to both test tubes and the absorbance of the sample was read against the sample blank after 10 min.

Calculation for TBIL: mg/dL = 10.8 x absorbance value

3.9.4.2 Direct bilirubin (DBIL)

0.2 ml of reagent 1, 2 ml of normal saline and 0.2 ml of the sample were mixed together in a test tube (sample blank) and allowed to stand for 5 min. In another test tube (sample), 0.2 ml of reagent 1, 1 drop of reagent 2, 2 ml of normal saline and 0.2 ml of the sample were mixed together and also allowed to stand for 5 min, after which the absorbance of the sample was read against the sample blank.

Calculation for DBIL: mg/dL = 14.4 x absorbance value

3.9.5 Cholesterol (colorimetric – enzymatic end point method)

Reagent: 4-aminoanipyrine 0.3 mmol/L

Phenol 6 mmol/L

Peroxidase > 0.5 μ/ml

Cholesterol esterase > 0.15 μ/ml

Cholesterol oxidase > 0.1 μ/ml

Pipes buffer 80 mmol/L pH 6.8

Standard: 5.17 mmol/L (200 mg/dL)

Sample: Serum

Cuvette: 1 cm light path

Wavelength (λ): 500, 546 nm

10 μL of distilled water, 10 μL of the standard and 10 μL of the sample were pipette into the reagent blank, standard and sample test tubes respectively, followed by 1000 μL of the reagent into each of the three tubes. The test tubes were mixed and incubated for 10 min at 20-25^{o}C or 5 min at 37^{o}C. The absorbances of the sample and standard were measured against the reagent blank within 60 min.

Calculation for cholesterol: mmol/L = $\frac{\text{A sample}}{\text{A standard}}$ x 5.17 (Std. conc.)

3.9.6 Triglycerides (colorimetric method)

Reagent: 4-aminophenazone 0.5 mmol/L

ATP 1mmol/L

Lipases > 150 μ/ml

Glycerol kinase > 0.4 μ/m

Glycerol-3-phosphate oxidase . 1.5 μ/ml

Peroxidase > 0.5 μ/ml

Pipes buffer 40 mmol/L

4-chlorophenol 5.5 mmol/L

Magnesium ions 17.5 mmol/L

Standard: 2.29 mmol/L (200 mg/dL)

Sample: Serum

Cuvette: 1 cm light path

Wavelength (λ): 500, 546 nm

1000 µL of the reagent was pipette into each of the three test tubes used for the assay (reagent blank, standard and sample test tubes), followed by 10 µL of the standard and 10 µL of the sample into the standard and sample tubes respectively. The test tubes were mixed and incubated for 10 min at 20-25^{o}C or 5 min at 37^{o}C. The absorbances of the sample and standard were measured against the reagent blank.

Calculation for triglycerides: mmol/L = $\frac{\text{A sample}}{\text{A standard}}$ x 2.29

3.9.7 Albumin (colorimetric method)

Reagent: Bromocresol green 0.26 mmol/L

Citrate buffer, pH 4.2, 30 mmol/L

Standard: 5 g/dL

Sample: Serum

Cuvette: 1 cm light path

Wavelength (λ): 546 nm, 540-600 nm

1000 µL of the reagent was pipette into each of the three test tubes used for the assay (reagent blank, standard and sample test tubes), followed by 10 µL of the standard and 10 µL of the sample into the standard and sample tubes respectively. The test tubes

were mixed and incubated for 10 min at 20-25^{o}C/37^{o}C. Absorbances of the sample and standard were measured against the reagent blank within 60 min.

Calculation for albumin: g/dL = $\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}}$ x 5 (conc. of standard)

g/L = g/dL x 10

3.10 Histopathological Examination

The liver was isolated and weighed (Matsuda *et al.*, 1991), then fixed in 10% formal saline (10%$^{v}/_{v}$ formaldehyde in normal saline) and sent out for histopathological examination to determine the extent of hepatocyte damage (positive control) and regeneration (extract treated).

3.11 Statistical Analysis

The results were expressed as mean ± standard error of the mean (SEM) [and percentages where appropriate]. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Student's t-test, using graph pad prism 4.0. P values < 0.05 were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction

Solid extracts of different consistencies were obtained from the three plants. *B. aegyptiaca* (B.A) yielded a brown, sticky, sweet smelling residue which was completely soluble in water. *B. dalzielii* (B.D) extract was a brownish-black, water soluble solid, while *I. asarifolia* (I.A) yielded a green, oily, semi-solid only partially soluble in water. Yields of 15.81%($^{w}/_{w}$) (*Balanites aegyptiaca*), 3.72% ($^{w}/_{w}$) (*Boswellia dalzielii)* and 6% ($^{w}/_{w}$) (*Ipomoea asarifolia)* were obtained. Quantities of the extracts obtained from the bulk extraction were sufficient for the entire study.

4.2 Phytochemical Screening

Phytochemical screening revealed the presence of several constituents in each extract. B.A and B.D were both found to contain alkaloids, saponins, flavonoids, terpenes, tannins and sterols, resins and volatile oils, while I.A revealed the presence of alkaloids, glycosides, flavonoids, terpenes, sterols, resins, phenols and volatile oils (Table 4.1).

Table 4.1: Phytochemical constituents of *Balanites aegyptiaca* (B.A), *Boswellia dalzielii* (B.D) and *Ipomoea asarifolia* (I.A)

Phytochemical Constituents	**Plants Screened**		
	B.A	**B.D**	**I.A**
Alkaloids	+	+	+
Saponins	+	+	_
Tannins	+	+	_
Glycosides	_	_	+
Flavonoids	+	+	+
Terpenes	+	+	+
Sterols	+	+	+
Resins	+	+	+
Phenols	_	_	+
Volatile oils	+	+	+

Key: +:- present; - :- absent

4.3 LD_{50} Determination and Acute Toxicity Studies

The oral median lethal dose (LD_{50}) for the three extracts was found to be above 2000mg/kg as no mortality was observed at this dose. The therapeutic dose was thus determined to be 200 mg/kg ($^{1}/_{10}^{th}$ of LD_{50}), while the two other doses used for the study were 400 mg/kg and 100 mg/kg. Signs of neurological toxicity such as writhing, twitching, restlessness and panting were observed in I.A-treated animals at 300 mg/kg b.wt, while twitching was observed in rats given B.D at 300 mg/kg b.wt. No behavioural changes were observed after administration of B.A at the same dose. At 2000 mg/kg b.wt, behavioural changes characterised by writhing, constricted pupils, trembling, and lethargy were seen in I.A-treated animals, while constricted pupils, lethargy and jerking were observed in B.D-treated animals. Administration of B.A at the same dose resulted in lethargy and sleep, which had an onset of 30mins and lasted for about 15mins.

4.4 Hepatoprotective Studies

4.4.1 Direct method

4.4.1.1 Prophylactic study

I. Effects of methanolic extracts of *B. aegyptiaca, B. dalzielli, I. asarifolia* and their mixture (MIX) on biomarkers of liver injury in CCl_4-induced hepatotoxic rats:

Methanolic crude extracts of B.A, B.D and I.A significantly ($P<0.0001$) reduced elevated levels of serum AST and ALT observed in the CCl_4 treated group, offering a much better protection than silymarin at 100 mg/kg ($P<0.0001$). B.A was found to be most effective at 200 mg/kg, producing 76.00% and 79.55% reduction in AST and ALT levels respectively. B.D showed a dose-dependent protection, with 400 mg/kg offering 58.88% AST and 72.09% ALT reduction. Protection with I.A, was seen to decrease as the dose was increased, thus 100 mg/kg provided the highest reduction of AST (71.39%) and ALT (80.49%) levels. MIX on the other hand, offered no protection at all, showing a significant increase in toxicity. ALP levels were seen to decrease significantly in all extract treated groups including MIX, with B.D showing the least protection (21.62%; $P<0.05$) and silymarin offering the highest reduction (73.29%; $P<0.0001$) when compared to CCl_4 treated group. Only 400 mg/kg B.D and 100 mg/kg I.A showed significant decrease ($P<0.05$) in serum levels of direct bilirubin (DBIL) and total bilirubin (TBIL) compared to the toxin treated group (Table 4.2).

Table 4.2: Prophylactic effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on biomarkers of liver injury in carbon tetrachloride-induced hepatotoxic rats

	AST (u/L)	**ALT (u/L)**	**ALP (u/L)**	**DBIL (mmol/L)**	**TBIL (mmol/L)**
Control	134.00±6.71	75.17±2.89	134.30±4.65	1.43±0.14	2.70±0.21
Toxin (CCl_4)	1210.00±5.78^{+++}	891.00±7.13^{+++}	319.30±8.69^{+++}	2.20±0.35^{+}	6.64±1.02^{+}
B.A 100mg/kg + CCl_4	1088.00±3.25*** (11.34)	765.20±7.34*** (15.42)	233.50±2.01*** (46.38)	1.72±0.15	5.06±0.42
B.A 200mg/kg + CCl_4	392.20±6.34*** (76.00)	242.00±7.82*** (79.55)	252.70±2.99*** (36.00)	1.46±0.14	4.38±0.38
B.A 400mg/kg + CCl_4	487.30±6.28*** (67.17)	359.20±6.93*** (65.19)	187.20±5.57*** (71.40)	1.84±0.07	5.56±0.22
B.D 100mg/kg + CCl_4	813.70±6.97*** (36.83)	562.00±6.35*** (40.33)	279.30±4.97* (21.62)	2.12±0.32	6.37±0.98
B.D 200mg/kg + CCl_4	730.80±8.98*** (44.54)	468.30±5.95*** (51.81)	304.30±7.38	1.97±0.25	5.90±0.75
B.D 400mg/kg + CCl_4	576.50±3.60*** (58.88)	302.80±2.82*** (72.09)	263.00±9.29* (30.43)	1.38±0.14* (106.49)	4.17±0.41* (62.69)
I.A 100mg/kg + CCl_4	441.80±5.43*** (71.39)	233.50±6.95*** (80.49)	199.30±4.74*** (64.86)	1.40±0.12* (103.89)	4.22±0.40* (61.42)
I.A 200mg/kg + CCl_4	516.20±8.06*** (64.48)	332.20±9.87*** (68.49)	242.00±9.38** (41.78)	1.70±0.15	5.12±0.45
I.A 400mg/kg + CCl_4	663.20±8.63*** (50.82)	558.30±8.51*** (40.78)	261.80±9.89* (31.08)	1.78±0.13	5.42±0.41
MIX + CCl_4	1267.00±8.60** (-5.29)	1173.00±7.52*** (-34.57)	268.50±3.00** (27.46)	3.40±0.15* (-155.84)	6.72±0.29
Silymarin 100mg/kg+CCl_4	756.70±5.14*** (42.13)	647.20±4.51*** (29.88)	183.70±4.99*** (73.29)	2.30±0.22	5.30±0.75

Values are mean ± S.E.M, n=6. Values in parentheses indicate percentage protection. Values are statistically significant at ***P<0.0001, **P<0.001, *P<0.05 compared to toxin group; and $^{+++}$P<0.0001, $^{++}$P<0.001, $^{+}$P<0.05 compared to control (ANOVA followed by Student's t-test)

II. Effects of methanolic extracts of *B. aegyptiaca, B. dalzielli, I. asarifolia* and their mixture (MIX) on lipid profile, albumin and liver weight in CCl_4-induced hepatotoxic rats:

B. aegyptiaca and *I. asarifolia* showed significant ($P<0.0001$) reduction in serum triglyceride levels comparable to that of 100 mg/kg silymarin (92.30%), at doses of 400 mg/kg B.A (98.76%) and 100 mg/kg I.A (62.96%). B.D offered a lesser protection ($P<0.001$) at 200 mg/kg (55.19%). 200 mg/kg B.A, 400 mg/kg B.D and 200 mg/kg I.A produced a significant ($P<0.05$) increase in serum albumin levels (96.91%) better than silymarin ($P>0.05$). Only B.A and I.A showed a significant ($P<0.05$) decrease in cholesterol levels when compared to the toxin-treated group. MIX showed a significant ($P<0.0001$) decrease only in triglyceride levels, compared to other indices ($P>0.05$).

A significant ($P<0.05$) increase in liver weight was observed in the CCl_4 treated group when compared to the control group. This was significantly ($P<0.05$) reduced in all extract treated groups except MIX. B.A showed a dose-dependent reduction, with 400 mg/kg offering the highest protection (47.31%), whereas B.D and I.A produced a reduction in liver weight which was not dose-dependent at 400 mg/kg (58.06%) and 100 mg/kg (29.03%) respectively. The effect on liver weight produced by the standard drug silymarin was not significant (Table 4.3).

Table 4.3: Prophylactic effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on lipid profile, serum albumin and liver weight in carbon tetrachloride-induced hepatotoxic rats

	CHOL (mmol/L)	**TRIG (mmol/L)**	**ALB (g/L)**	**LW**
Control	67.00±1.24	78.17±4.52	34.67±0.49	3.90±0.30
Toxin (CCl_4)	83.17±2.96^{++}	145.2±3.18^{+++}	29.17±1.28^{+}	4.83±0.11^{+}
B.A 100mg/kg + CCl_4	67.67±2.80* (95.86)	80.33±2.64*** (96.78)	32.50±1.18	4.48±0.11* (37.63)
B.A 200mg/kg + CCl_4	78.67±8.43	123.70±4.31* (32.08)	34.50±1.43* (96.91)	4.44±0.11* (41.94)
B.A 400mg/kg + CCl_4	67.83±1.85* (94.87)	79.00±1.53*** (98.76)	29.50±0.81	4.39±0.07* (47.31)
B.D 100mg/kg + CCl_4	75.33±2.77	116.00±8.22* (43.56)	33.50±0.99* (78.73)	4.67±0.11
B.D 200mg/kg + CCl_4	75.17±4.87	108.20±6.02** (55.19)	31.83±1.45	4.64±0.18
B.D 400mg/kg + CCl_4	84.67±3.29	112.30±4.33** (49.08)	34.50±0.43* (96.91)	4.29±0.11* (58.06)
I.A 100mg/kg + CCl_4	67.67±3.54* (95.86)	103.00±2.44*** (62.96)	33.50±0.43* (78.73)	4.56±0.04* (29.03)
I.A 200mg/kg + CCl_4	69.00±3.71* (87.63)	115.20±5.51** (44.76)	34.50±0.67* (96.91)	4.77±0.10
I.A 400mg/kg + CCl_4	69.17±3.16* (86.58)	148.50±5.95	32.67±0.80* (63.64)	4.79±0.20
MIX + CCl_4	85.00±4.52	102.20±3.25*** (64.15)	27.00±1.55	4.75±0.15
Silymarin 100mg/kg + CCl_4	70.33±2.47* (79.41)	83.33±3.82*** (92.30)	30.00±1.13	4.88±0.19

Values are mean ± S.E.M, n=6. Values in parentheses indicate percentage protection. Values are statistically significant at ***P<0.0001, **P<0.001, *P<0.05 compared to toxin group; and $^{+++}$P<0.0001, $^{++}$P<0.001, $^{+}$P<0.05 compared to control (ANOVA followed by Student's t-test)

4.4.1.2 Curative study

I. Effects of methanolic extracts of *B. aegyptiaca*, *B. dalzielli*, *I. asarifolia* and their mixture (MIX) on biomarkers of liver injury in CCl_4-induced hepatotoxic rats:

Serum levels of the hepatic enzymes AST, ALT and ALP were significantly ($P<0.0001$) increased in the toxin treated group when compared to the control. The three extracts and their mixture produced significant reduction in AST levels, with I.A and B.A showing significant hepatoprotection (88.24% at 400 mg/kg and 97.96% at 200 mg/kg respectively). B.D (100 mg/kg) produced lesser protection (21.47%) comparable to that of 100 mg/kg silymarin (21.78%). Significant reduction in serum ALT levels was observed in silymarin, MIX, B.A and 100 mg/kg B.D treated groups. Silymarin, B.D and B.A caused significant ($P<0.0001$) reduction in serum ALP levels, while I.A (400 mg/kg) showed a significant ($P<0.001$) increase in toxicity, greater than that observed in the toxin group. DBIL and TBIL were dose-dependently reduced by I.A, producing much lower values (151.52% and 133.48%, respectively) at 400 mg/kg than that observed in the control. Effects of other extracts including silymarin were not significant on serum DBIL levels and only B.D (200 and 400 mg/kg) showed significant ($P<0.05$) reduction in TBIL levels compared to toxin group (Table 4.4).

Table 4.4: Curative effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on biomarkers of liver injury in carbon tetrachloride-induced hepatotoxic rats

	AST (u/L)	**ALT (u/L)**	**ALP (u/L)**	**DBIL (mmol/L)**	**TBIL (mmol/L)**
Control	134.00±6.71	75.17±2.89	134.30±4.65	1.43±0.14	2.70±0.21
Toxin (CCl_4)	231.80±2.66^{+++}	116.80±4.28^{+++}	204.50±2.22^{+++}	2.42±0.22^{+}	5.03±0.33^{+}
B.A 100mg/kg + CCl_4	169.40±4.26*** (63.80)	84.40±3.42** (77.82)	160.80±5.54*** (62.25)	1.94±0.18	4.14±0.39
B.A 200mg/kg + CCl_4	136.00±5.85*** (97.96)	85.50±7.96* (75.19)	163.80±5.54*** (57.98)	2.03±0.29	4.00±0.53
B.A 400mg/kg + CCl_4	225.00±5.52	89.50±4.27* (65.58)	149.80±5.49*** (77.92)	2.07±0.22	4.07±0.43
B.D 100mg/kg + CCl_4	210.80±3.57* (21.47)	100.30±3.09* (39.63)	181.20±4.32** (33.19)	1.70±0.42	3.42±0.82
B.D 200mg/kg + CCl_4	219.20±1.59* (12.88)	106.40±6.81	202.20±5.27	1.76±0.30	3.54±0.57* (63.95)
B.D 400mg/kg + CCl_4	253.00±3.76* (-21.68)	114.50±7.30	163.50±4.98*** (58.40)	1.80±0.24	3.53±0.57* (64.38)
I.A 100mg/kg + CCl_4	174.60±5.71*** (58.49)	107.00±7.82	197.30±3.90	1.95±0.41	3.90±0.76
I.A 200mg/kg + CCl_4	172.60±5.80*** (60.53)	111.80±4.09	195.6±9.59	1.35±0.13* (108.08)	2.85±0.25* (93.56)
I.A 400mg/kg + CCl_4	145.50±5.64*** (88.24)	123.60±4.86	222.40±2.44** (-25.50)	0.92±0.14** (151.52)	1.92±0.25* (133.48)
MIX + CCl_4	171.00±7.69** (62.17)	92.17±3.04* (59.16)	152.30±2.02*** (74.36)	2.38±0.34	4.88±0.65
Silymarin 100mg/kg+CCl_4	210.50±2.53* (21.78)	100.00±3.19* (40.63)	151.80±3.43*** (75.07)	2.37±0.29	4.43±0.75

Values are mean ± S.E.M, n=6. Values in parentheses indicate percentage recovery. Values are statistically significant at ***P<0.0001, **P<0.001, *P<0.05 compared to toxin group; and $^{+++}$P<0.0001, $^{++}$P<0.001, $^{+}$P<0.05 compared to control (ANOVA followed by Student's t-test)

II. Effects of methanolic extracts of *B. aegyptiaca, B. dalzielli, I. asarifolia* and their mixture (MIX) on lipid profile, albumin and liver weight in CCl_4-induced hepatotoxic rats:

Table 4.5 shows the effects of B.A, B.D, I.A and MIX on lipid profile, albumin and liver weight. Only 400mg/kg B.A showed a significant ($P<0.05$) decrease in cholesterol level (94.64%). Serum levels of triglycerides were significantly reduced by MIX, B.A (200 mg/kg), B.D (200 mg/kg) and I.A (200 and 400 mg/kg), when compared to CCl_4 treated group. Silymarin (100 mg/kg) produced the most significant ($P<0.0001$) reduction in serum triglyceride level (103.57%), while B.D (400 mg/kg) produced a significant ($P<0.001$) increase in triglyceride level. The increase in serum albumin observed in toxin group was significantly ($P<0.05$) reduced in all treatment groups except B.D 400 mg/kg and B.A 400 mg/kg groups. No significant changes in liver weight were observed in any of the treatment groups except the silymarin group, where 100 mg/kg produced a 53.85% protection, when compared to the toxin group.

Table 4.5: Curative effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on lipid profile, albumin and liver weight in carbon tetrachloride-induced hepatotoxic rats

	CHOL (mmol/L)	**TRIG (mmol/L)**	**ALB (g/L)**	**LW**
Control	67.00±1.24	78.17±4.52	34.67±0.49	3.90±0.30
Toxin (CCl_4)	81.00±4.70^{+}	125.00±3.54^{+++}	28.20±0.73^{+++}	4.68±0.06^{+}
B.A 100mg/kg + CCl_4	72.40±1.86	125.00±2.52	32.00±1.27* (58.73)	4.59±0.04
B.A 200mg/kg + CCl_4	68.25±3.25	102.50±3.93* (48.05)	31.25±0.63* (47.14)	4.36±0.14
B.A 400mg/kg + CCl_4	67.75±1.44* (94.64)	122.00±2.45	32.33±2.19	4.62±0.17
B.D 100mg/kg + CCl_4	87.33±6.23	131.00±2.12	32.00±0.71* (58.73)	4.16±0.24
B.D 200mg/kg + CCl_4	83.00±4.20	109.20±5.86* (33.74)	31.80±1.56* (55.64)	4.33±0.14
B.D 400mg/kg + CCl_4	73.83±3.99	159.80±4.60** (-74.31)	30.00±1.37	4.33±0.16
I.A 100mg/kg + CCl_4	74.20±4.89	118.50±6.01	33.60±0.98* (83.46)	4.48±0.17
I.A 200mg/kg + CCl_4	73.80±3.48	78.75±8.83* (98.76)	33.83±0.95* (87.02)	4.34±0.14
I.A 400mg/kg + CCl_4	74.67±4.10	84.20±4.32*** (87.12)	33.33±1.93* (79.29)	4.50±0.11
MIX + CCl_4	76.17±3.25	81.00±6.37** (93.96)	32.80±1.39* (71.09)	4.48±0.11
Silymarin 100mg/kg + CCl_4	73.50±7.40	76.50±2.02*** (103.57)	32.33±1.20* (63.83)	4.26±0.15* (53.85)

Values are mean ± S.E.M, n=6. Values in parentheses indicate percentage recovery. Values are statistically significant at ***P<0.0001, **P<0.001, *P<0.05 compared to toxin group; and $^{+++}$P<0.0001, $^{++}$P<0.001, $^{+}$P<0.05 compared to control (ANOVA followed by Student's t-test)

4.4.2 Indirect method

4.4.2.1 Prophylactic study

I. Effects of methanolic extracts of *B. aegyptiaca, B. dalzielli, I. asarifolia* and their mixture (MIX) on PST in CCl_4-induced hepatotoxic rats:

Table 4.6 shows significant ($P<0.05$) prolongation of PST in toxin treated group when compared to control. B.A was seen to produce a significant dose-dependent increase in PST ($P<0.0001$) beyond that observed in the toxin group. B.D also produced a dose-dependent increase in PST, which was significant ($P<0.05$) at 400 mg/kg. I.A on the other hand showed a dose-dependent decrease in PST compared to toxin group, significant ($P<0.0001$) at 200 mg/kg (51.43%) and 400 mg/kg (60.83%). MIX produced a significant ($P<0.0001$) increase in PST comparable to that seen in B.A groups, while silymarin produced significant ($P<0.05$) reduction in PST (12.02%). B.A (200mg/kg and 400 mg/kg) produced a significant reduction ($P<0.05$) in onset of sleep, much less than that produced by the toxin group, while I.A (400 mg/kg) caused a significant increase ($P<0.05$) in onset of sleep, higher than the control.

Table 4.6: Prophylactic effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on pentobarbitone sleeping time in carbon tetrachloride-induced hepatotoxic rats

	Dose (mg/kg)	**Onset of Sleep (min)**	**Duration of Sleep/PST (min)**	**Recovery (%)**
Control		6.20±0.49	68.33±3.79	
Toxin (CCl_4)		4.83±0.48	164.00±4.62^{+}	
B.A + CCl_4	100	4.60±0.40	266.20±6.22***	-106.83
B.A + CCl_4	200	3.25±0.25*	273.70±8.37***	-114.66
B.A + CCl_4	400	3.40±0.24*	314.30±5.99***	-157.10
B.D + CCl_4	100	4.40±0.40	161.50±5.27	2.61
B.D + CCl_4	200	5.00±0.63	174.00±4.76	-10.45
B.D + CCl_4	400	5.00±1.00	186.20±2.55*	-23.20
I.A + CCl_4	100	4.60±0.24	156.20±9.11	8.15
I.A + CCl_4	200	5.40±0.75	114.80±5.81***	51.43
I.A + CCl_4	400	8.75±1.11*	105.80±7.77***	60.83
MIX + CCl_4		5.33±0.76	255.00±8.49***	-95.12
Silymarin + CCl_4	100	5.60±0.51	152.50±1.20*	12.02

Values are expressed as mean ± S.E.M [n=6] and are statistically significant at ***P<0.0001, *P<0.05 compared to toxin group; and $^{+}$P<0.05 compared to control (ANOVA followed by Student's t-test)

4.4.2.2 Curative Study

I. Effects of methanolic extracts of *B. aegyptiaca, B. dalzielli, I. asarifolia* and their mixture (MIX) on PST in CCl_4-induced hepatotoxic rats:

Table 4.7 shows that all doses of B.A caused significant ($P<0.05$) reduction in PST compared to the toxin group, with 200 mg/kg offering the highest reduction (86.23%). 100 and 200 mg/kg B.D also produced a significant reduction in PST (88.27% and 90.62% respectively) which was comparable to that of B.A; while 400 mg/kg B.D, MIX and all doses of I.A showed significant reductions in PST lower than that observed in the control group. There was no significant change in PST observed in the silymarin group. Effect of the extracts and silymarin on onset of sleep was not significant, except in I.A (400 mg/kg) and MIX treated groups, both of which produced significant ($P<0.05$) increase in sleep onset.

Table 4.7: Curative effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on pentobarbitone sleeping time in carbon tetrachloride-induced hepatotoxic rats

	Dose (mg/kg)	**Onset of Sleep (min)**	**Duration of Sleep/PST (min)**	**Recovery (%)**
Control		6.20±0.49	68.33±3.79	
Toxin (CCl_4)		5.50±0.50	96.80±3.34^{++}	
B.A + CCl_4	100	4.00±1.00	81.40±4.62*	54.09
B.A + CCl_4	200	7.25±1.11	72.25±8.41*	86.23
B.A + CCl_4	400	6.25±0.75	81.50±3.97*	53.74
B.D + CCl_4	100	6.20±0.37	71.67±9.49*	88.27
B.D + CCl_4	200	7.60±0.93	71.00±4.57*	90.62
B.D + CCl_4	400	7.17±0.60	67.00±3.44**	104.67
I.A + CCl_4	100	5.40±0.40	63.67±6.89*	116.37
I.A + CCl_4	200	7.40±0.60	55.00±7.89*	146.82
I.A + CCl_4	400	7.40±0.51*	64.33±7.15*	114.05
MIX + CCl_4		10.75±1.60*	34.00±1.63***	220.58
Silymarin + CCl_4	100	5.00±0.78	89.00±11.59	27.40

Values are expressed as mean ± S.E.M [n=6] and are statistically significant at ***P<0.0001, **P<0.001, *P<0.05 compared to toxin group; and $^{++}$P<0.001 compared to control (ANOVA followed by Student's t-test)

4.5 Histopathological Examination

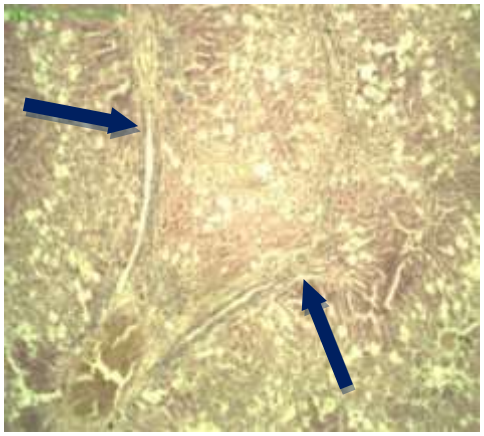
4.5.1 Prophylactic study

I. Effects of methanolic extracts of *B. aegyptiaca, B. dalzielli, I. asarifolia* and their mixture (MIX) on liver pathology in CCl_4-induced hepatotoxic rats:

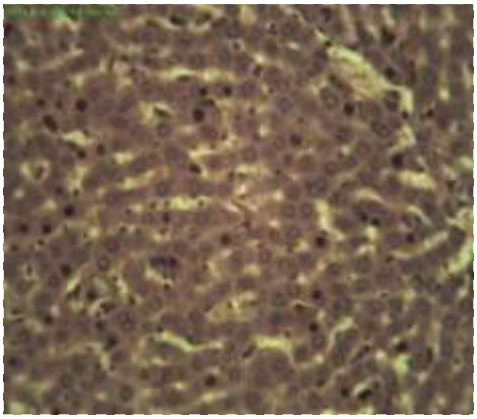
Histopathological profile of group I (control) animals showed normal hepatic architecture (Plate Ia), while CCl_4 treated animals exhibited severe hepatotoxicity, indicated by marked fibrosis (Plate Ib), marked fatty change and moderate necroinflammation with congested vessels. All extract treated and silymarin groups showed reduction in hepatic damage, collaborating results obtained from biochemical analysis. B.A (400 mg/kg) and B.D (100 mg/kg) demonstrated the most significant improvement, producing only mild focal fibrosis and mild fatty change with normal portal tract architecture (Plate Ic). Liver sections of I.A (200 mg/kg) showed mild fibrosis, mild fatty change (Plate Ie) and moderate peri-portal necroinflammation, while silymarin treated animals exhibited mild fibrosis, moderate fatty change and mild peri-portal inflammation (Plate Id). MIX treated group however, showed lesser protection compared to the individual extract treated groups, in the form of moderate fibrosis, moderate fatty change (Plate If) and moderate peri-portal inflammation. Table 4.8 shows a summary of histopathological changes observed in all the groups.



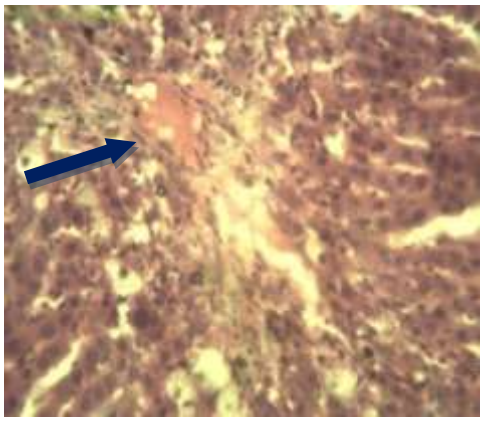
(a) Normal Liver x100



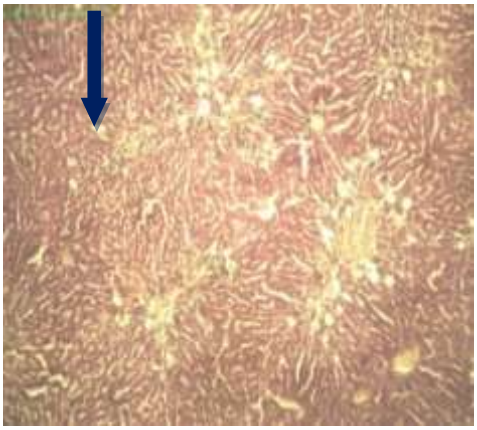
(b) Marked fibrosis x100



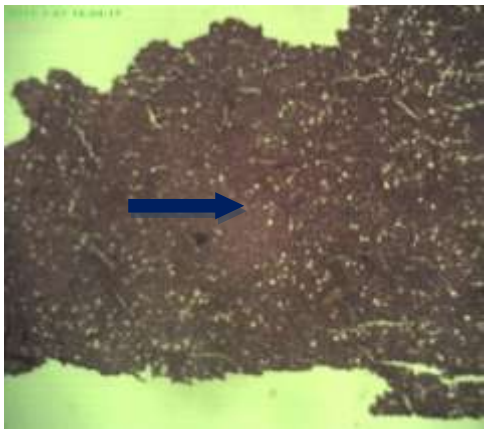
(c) Normal Portal Tract x400



(d) Peri-portal Inflammation x400



(e) Mild Fatty Change x100



(f) Moderate Fatty Change x40

Plate I: Photomicrographs of liver sections of (a): control; (b): carbon tetrachloride treated group; (c): B.A (400 mg/kg) and B.D (100 mg/kg) treated groups; (d): silymarin treated group; (e): I.A (200 mg/kg) treated group (f): MIX treated group

Labels are written as: description x magnification; Staining Technique: Haemotoxylin and Eosin Stain

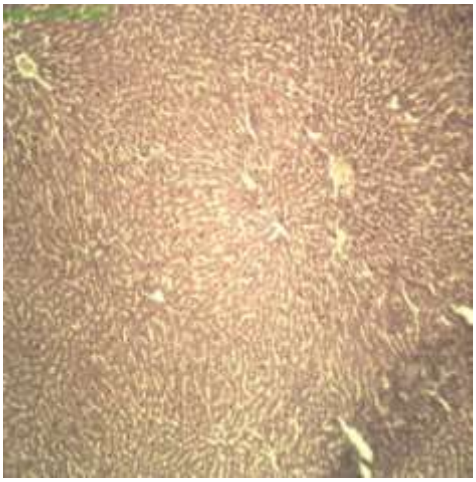
Table 4.8: Prophylactic effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on histopathological changes observed in carbon tetrachloride-induced hepatotoxic rats

	Parenchyma	**Hepatocytes**	**Portal tract**
Control	-	-	-
Toxin (CCl_4)	Marked fibrosis	Marked fatty change	Moderate necroinflammation and congested vessels
B.A 400mg/kg +CCl_4	Mild focal fibrosis	Mild fatty change	-
B.A 200mg/kg +CCl_4	Mild fibrosis	Moderate fatty change	Moderate peri-portal necroinflammation
B.A 100mg/kg +CCl_4	Marked fibrosis	Moderate fatty change	Moderate peri-portal necroinflammation
B.D 400mg/kg +CCl_4	Mild focal fibrosis	Mild fatty change	Moderate peri-portal necroinflammation
B.D 200mg/kg +CCl_4	Moderate fibrosis	Moderate fatty change	Moderate necroinflammation
B.D 100mg/kg +CCl_4	Mild fibrosis	Mild fatty change	-
I.A 400mg/kg +CCl_4	Moderate fibrosis	Marked fatty change	Moderate necroinflammation
I.A 200mg/kg +CCl_4	Mild fibrosis	Mild fatty change	Moderate peri-portal necroinflammation
I.A 100mg/kg +CCl_4	Moderate fibrosis	Moderate fatty change	Mild necroinflammation
MIX +CCl_4	Moderate fibrosis	Moderate fatty change	Moderate peri-portal inflammation
Silymarin 100mg/kg+CCl_4	Mild fibrosis	Moderate fatty change	Mild peri-portal inflammation

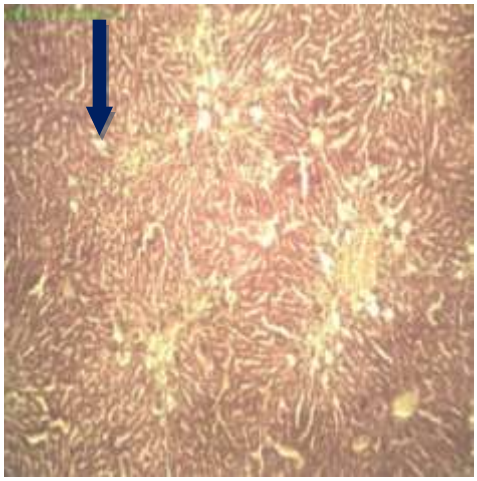
4.5.2 Curative study

I. Effects of methanolic extracts of *B. aegyptiaca, B. dalzielli, I. asarifolia* and their mixture (MIX) on liver pathology in CCl_4-induced hepatotoxic rats:

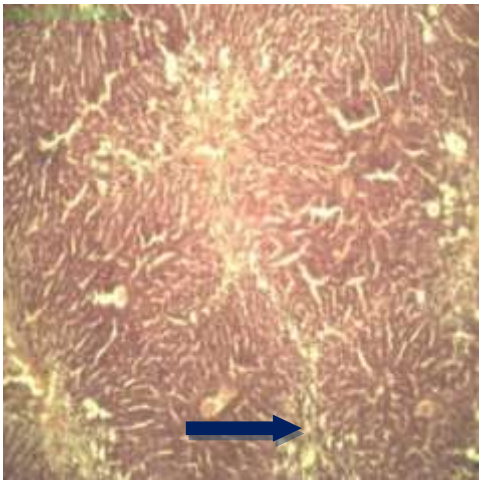
Table 4.9 shows the different histopathological changes observed in all the experimental groups. The toxin treated group exhibited liver damage in the form of mild fatty change (Plate IIb). All treatment groups reversed this fatty change to normal hepatic architecture (Plate IIa). However, B.D (400 mg/kg) exhibited greater damage (Plate IIc) than that observed in the toxin group, while I.A (400 mg/kg) produced an even greater damage, presenting as moderate fibrosis, moderate fatty change and moderate necroinflammation (Plate IId).



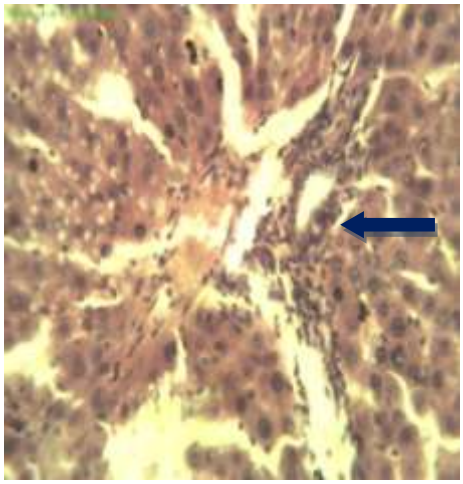
(a) Normal Liver x100



(b) Mild Fatty Change x100



(c) Mild Fibrosis x100



(d) Moderate Necroinflammation x400

Plate II: Photomicrographs of liver sections of (a): normal liver; (b): carbon tetrachloride treated group; (c): B.D (400 mg/kg) treated group; (d): I.A (400 mg/kg) treated group

Labels are written as: description x magnification; Staining Technique: Haemotoxylin and Eosin Stain

Table 4.9: Curative effects of *Balanites aegyptiaca* (B.A), *Boswellia dalzielli* (B.D), *Ipomoea asarifolia* (I.A) and their mixture (MIX) on histopathological changes observed in carbon tetrachloride-induced hepatotoxic rats

	Parenchyma	**Hepatocytes**	**Portal tract**
Control	-	-	-
Toxin (CCl_4)	-	Mild fatty change	-
B.A 400mg/kg +CCl_4	-	-	-
B.A 200mg/kg +CCl_4	-	-	-
B.A 100mg/kg +CCl_4	-	-	-
B.D 400mg/kg +CCl_4	Mild fibrosis	-	Mild Inflammation
B.D 200mg/kg +CCl_4	-	-	-
B.D 100mg/kg +CCl_4	-	-	-
I.A 400mg/kg +CCl_4	Moderate fibrosis	Moderate fatty change	Moderate necroinflammation
I.A 200mg/kg +CCl_4	-	-	-
I.A 100mg/kg +CCl_4	-	-	-
MIX + CCl_4	-	-	-
Silymarin 100mg/kg +CCl_4	-	-	-

A comparison of the toxin groups in the prophylactic and curative studies revealed that in the prophylactic study, the CCl_4-treated group showed marked liver damage characterized by extensive fibrosis, marked fatty liver and congested vessels (Table 4.8), while the toxin group in the curative study presented with only mild fatty change (Table 4.9).

CHAPTER FIVE

5.0 DISCUSSION

The liver is the largest, most complex internal organ, which has a remarkably unique inherent capacity to fully repair itself after significant hepatic tissue loss. The classical model of hepatic regeneration is that of partial hepatectomy in which approximately 70% of the liver is resected and the remaining lobes regenerate to the original size of the liver which takes 5-7 days in the rat (Ankoma-Sey, 1999). The liver plays a pivotal role in maintenance of carbohydrate homeostasis, nutrient storage, secretory and excretory functions, protein synthesis and metabolic processes such as hormone, lipid, and protein metabolism and detoxification of xenobiotics (Guyton, 1981). It is involved in almost all biochemical pathways related to growth, fight against disease, nutrient supply, energy production and reproduction (Ward and Daly, 1999). Most of these biochemical reactions are catalysed by enzymes (such as AST and ALT) present in the hepatocytes. In the presence of hepatic injury, such as that produced by hepatotoxins, the transport function of the cells is impaired, leading to a leakage of these enzymes from the cell cytoplasm into serum (Usha et al, 2008). This increase in serum transaminase levels is a clear indication of hepatocellular damage and loss of functional integrity of the cell membranes.

Carbon tetrachloride (CCl_4) is one of the most potent hepatotoxins and is thus widely used in the experimental study of liver diseases (Johnson and Kroening, 1998). It induces acute liver damage in the form of oxidative-stress related hepatitis following its enzymatic activation by cytochrome P450 to produce free radicals within the membranes of the endoplasmic reticulum. The free radicals (e.g. trichloromethyl (CCl_3), trichloromethyl peroxy (CCl_3OO), hexachloroethane ($Cl_3C\text{-}CCl_3$) and phosgene

($COCl_2$)) combine with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation, covalent binding to macromolecules, disruption of metabolic mechanisms in mitochondria, decrease in phospholipid levels, increased triglyceride levels and inhibition of the calcium pump of microsomes, leading to liver necrosis (Liu et al., 2005).

Hepatoprotective properties of the standard drug, silymarin, have been attributed to the presence of four bioactive constituents (flavanolignans) found in the dried seeds of the plant, *Silybum marianum*, commonly known as 'milk thistle' (Pradhan and Girish, 2006). Sharma *et al.*, (1991) reported that silymarin treatment led to complete normalization of elevated transaminases levels in CCl_4-induced hepatotoxic rats. Silymarin was also found to prevent cirrhotic changes and reduce liver collagen content by 55% when administered orally at a dose of 50 mg/kg b.wt for 5 days to rats with chronic CCl_4-induced liver damage (Favari and Perez-Alvarez, 1997). In another study, treatment of mice with silymarin at 100 mg/kg i.p. for 7 days, led to a reduction in phenobarbitone sleeping time (PST) and an increase in liver weight, twice that of the CCl_4 treated group. However, silymarin provided only 16% protection against CCl_4-induced hepatic necrosis in the same group of animals (Pradhan and Girish, 2006). Based on this information, a preliminary dose-response effect study was carried out on silymarin at doses of 50 mg/kg and 100 mg/kg, to determine the most effective dose for the study. 100 mg/kg silymarin was used as the standard in the study because it provided significant protection against CCl_4-induced liver damage, whereas hepatoprotection at 50 mg/kg was insignificant.

Many preclinical studies have shown that silymarin exerts its hepatoprotective activity via several mechanisms (Pradhan and Girish, 2006). In 1999, Renganathan suggested that silymarin may offer hepatoprotection by preventing hepatic cell necrosis or by hepatic cell regeneration. The antioxidant and cell-regenerating properties of silymarin, which occur as a result of increased protein synthesis, are considered the most important mechanisms of hepatoprotection (Kosina *et al.*, 2002). Other documented mechanisms of action include; anti-inflammatory properties resulting from inhibition of leukotriene synthesis (Saller *et al.*, 2001), anti-fibrotic activity (Gebhardt, 2002), regulatory action on cellular and mitochondrial membrane permeability in association with increased membrane stability against xenobiotic injury (Munter *et al.*, 1986) and prevention of toxin absorption into hepatocytes by occupying the binding sites and inhibiting many transport proteins at the membrane site (Faulstich *et al.*, 1980). Silibinin, a principal component of silymarin, has been shown to be remarkable in the treatment of mushroom (*Amanita phalloides*) poisoning by preventing the uptake of amatoxins by hepatocytes, thereby protecting undamaged hepatic tissue and stimulating DNA-dependent RNA polymerases, leading to an increase in RNA synthesis (Carducci *et al.*, 1996). Pradhan and Girish, (2006) also reported its usefulness in the management of some forms of liver disease, such as alcoholic liver disease, liver cirrhosis and viral hepatitis.

A series of experiments were conducted to ascertain the efficacy of *Balanites aegyptiaca* root (B.A), *Boswellia dalzielii* stem bark (B.D) and *Ipomoea asarifolia* (I.A) in the management of liver diseases. Preliminary studies included determining the choice of solvent for extraction, doses of extracts and dose of the standard drug used in the study. Acute oral toxicity studies were then carried out following the OECD-423

guideline. During which none of the three extracts resulted in animal mortality at 2000 mg/kg b.wt. However, the signs of neurological toxicity observed show that even though the extracts may be relatively safe when administered orally, dosage selection has to be carefully carried out to prevent adverse drug reactions. The OECD acute toxic class method is not intended to allow the calculation of a precise LD_{50} but an exposure range where lethality is expected. Therefore, based on the results obtained, the three extracts were placed in hazard category 5 of the Globally Harmonized Classification System (2000 mg/kg<LD_{50}<5000 mg/kg), indicating that the plant materials are of relatively low oral toxicity (OECD, 423). This provides a basis for safe oral administration of the plants and supports their traditional use as oral remedies.

Phytoconstituents such as flavonoids, saponins, alkaloids and triterpenes have been shown to act as heptoprotective agents by accelerating the excretion of CCl_4 or inhibiting CCl_4-induced lipid peroxidation (Mehta *et al.*, 1999). Flavonoids and tannins have also been reported to provide hepatoprotection by acting as antioxidants that scavenge the free radicals produced by the toxin (Chen *et al.*, 1990). Phytochemical screening of methanolic extracts of B.A, B.D and I.A revealed the presence of tannins, flavonoids, saponins, alkaloids, terpenes, glycosides and volatile oils, which may be responsible for their observed hepatoprotective properties. In agreement with these findings, other studies reported the presence of alkaloids and volatile oils in I.A (Kucht *et al.*, 2004); saponins and alkaloids in B.A (Yadav and Panghal, 2010) and alkaloids, tannins, saponins and anthraquinones in B.D (Etuk et al, 2006).

Administration of CCl_4 to experimental animals has been reported to significantly increase serum AST and ALT levels (Rajesh and Latha, 2004), supporting the increase

in serum enzyme levels observed in animals treated with CCl_4 in this study. The significant reduction in serum enzyme levels observed in all extract (B.A, B.D, and I.A)-treated rats indicate hepatoprotective activity. In CCl_4-induced liver injury, excretion of bile is also impaired, with consequent serum elevation of ALP and bilirubin, normally excreted by the liver via the bile. Increased levels of unconjugated bilirubin (TBIL-DBIL) observed could be due to inability of the damaged liver to conjugate the bilirubin, which is a prerequisite for bilirubin excretion (Nelson and Cox, 2000). The results of this study indicate a significant ($P<0.05$) rise in ALP, DBIL and TBIL levels in the CCl_4-treated group when compared to control, indicating hepatobiliary dysfunction and/or impaired bilirubin conjugation. All extract treated groups in the prophylactic and curative studies showed significant reduction in CCl_4-induced increase in serum ALP indicating possible hepatoprotection.

Prophylactic administration of the methanolic extracts of B.D (400 mg/kg) and I.A (100 mg/kg) produced significant reduction in TBIL and DBIL levels when compared to the CCl_4-treated group. This further corroborates the fact that B.D and I.A may act prophylactically to prevent biliary dysfunction in addition to improving the conjugating capacity of the liver. In the curative study, the significant reduction ($P<0.05$) in serum TBIL and DBIL levels observed in I.A (200 and 400 mg/kg) treated groups suggests a reversal of previously induced biliary dysfunction. In B.D (200 and 400 mg/kg) treated groups however, the elevated DBIL levels and near normal indirect (unconjugated) bilirubin levels produced indicate that the extract may have no effect on biliary dysfunction when administered after liver damage has occurred, but could improve hepatic conjugation by activating the constitutive androstane receptor (CAR) which is a key regulator in bilirubin clearance in the liver. According to Moore *et al.* (2004), CAR

acts primarily by directing a coordinate response to elevated levels of bilirubin, thereby increasing the hepatic expression of each component of the bilirubin clearance pathway.

Hepatotoxins reduce the capacity of the liver to synthesise proteins such as albumin, leading to a decrease in serum albumin level as observed in the CCl_4-treated rats. In the prophylactic study, administration of the individual extracts significantly ($P<0.05$) increased serum albumin level to near normal, even better than that observed in rats treated with silymarin. The curative study revealed a significant ($P<0.05$) increase in serum albumin levels in both the extract-treated groups and MIX-treated group, comparable to that of silymarin. This indicates that the extracts could be stimulating protein synthesis, which has been reported as a contributory hepatoprotective mechanism that accelerates regeneration of cells (Awang, 1993).

In the presence of liver damage, cholesterol levels tend to rise probably due to one or more of the following mechanisms; retention of cholesterol normally excreted in bile, regurgitation of biliary cholesterol into circulation and reduced plasma lecithin cholesterol acyl transferase (LCAT) activity. LCAT is the enzyme responsible for esterification of cholesterol (Sherlock and Dooley, 2002). Damage to the liver also interferes with apolipoprotein synthesis, leading to difficulty in transport of triglycerides as very low density lipoproteins (VLDL) and subsequent increase in serum triglyceride levels. This is responsible for the fatty liver seen on histopathological examination of the liver of CCl_4-induced hepatotoxic rats. The significant ($P<0.05$) decrease in serum cholesterol level produced by B.A and I.A in the

prophylactic study and by B.D at 400 mg/kg in the curative study, suggest that the extracts may improve both synthetic and metabolic activities of the liver.

Histopathological examination of liver sections showed normal hepatic cells, normal parenchyma and normal portal tract in the control group. Pretreatment with the three extracts offered significant protection to the liver by preventing the extensive liver damage observed in the toxin group, better than that produced by silymarin. On the other hand, curative treatment with the extracts and MIX resulted in a complete reversal of the mild liver damage produced by the toxin, to normal liver histology comparable to that of silymarin. These results confirm those of biochemical analysis, suggesting that the extracts could offer both hepatoprotection via cell membrane stabilization and antihepatotoxic effects by repairing already damaged hepatic tissue. Gross pathological examination further revealed that the significant liver enlargement observed in CCl_4-treated rats was significantly reduced in all extract treated groups, indicating hepatoprotection and antihepatotoxicity.

The differences in extent of liver damage observed in the prophylactic and curative CCl_4-treated groups could be attributed to the remarkable inherent ability of the liver to fully regenerate itself after significant hepatic tissue loss or acute liver injury. This would only be possible in the curative groups as CCl_4 administration was discontinued for 7 days prior to sacrifice, allowing enough time for regeneration; unlike in the prophylactic groups where CCl_4 was administered up to the last day prior to sacrifice. Curative treatments with B.D and I.A (400 mg/kg) on the other hand, resulted in an increase in histopathological damage when compared to the CCl_4-treated group. This damage is supported by an increase in serum levels of some biochemical parameters in

these groups, suggesting that the extracts (B.D and I.A) though hepatoprotective at low doses, may be hepatotoxic at higher doses. This implies a dose-dependent potential for both liver protection and hepatotoxicity. A number of mechanisms could be responsible for the observed hepatotoxic activity of the extracts, some of which may include increasing the metabolism of CCl_4 or preventing its excretion.

Pentobarbitone is largely metabolized in the liver by microsomal drug metabolizing enzymes (MDME) to inactive alcohol metabolites. The duration of pentobarbitone induced sleep in intact animals is thus considered a reliable index for hepatic MDME activity (Conney, 1967). Any substance that inhibits MDME is likely to prolong pentobarbitone sleeping time (Fujimoto *et al*., 1960), while any substance that induces the enzyme's activity is likely to shorten pentobarbitone sleeping time (PST). CNS depressants can also prolong PST without altering MDME activity (Shin, 1989) by increasing the activity of the neurotransmitter gamma-amino butyric acid (GABA) and inhibiting adrenergic, histamine and/or acetylcholine activity. Similarly, reduction in PST can be achieved by CNS stimulants through antagonism of inhibitory neurotransmission, altered pre-synaptic control of neurotransmitter release and enhanced dopamine and/or norepinephrine (noradrenaline) activity.

Prolongation of PST occurs in the presence of CCl_4-induced liver damage due to the destruction of the liver microsomal enzyme system. Substances that inhibit MDME can prevent activation of CCl_4 to its reactive metabolite thereby providing protection against hepatocellular damage (Castro *et al.*, 1974; Nelson *et al.*, 1980). This protection can only be achieved when the substance is administered before activation of the hepatotoxin to the reactive metabolites. The significant prolongation of PST following

prophylactic administration of B.A and B.D suggest that the extracts may provide hepatoprotection by inhibiting MDME, thereby preventing the conversion of CCl_4 to the free radicals responsible for liver toxicity. B.A and B.D may also prolong PST by depressing the CNS through one or more of the mechanisms mentioned above. In addition, the sedation and lethargy observed during acute toxicity studies with B.A and B.D further corroborate the CNS depressant properties of the extracts, which could also be responsible for the significant reduction in sleep onset observed in B.A treated groups. Treatment with the extract I.A on the other hand revealed a shortening of PST and a prolongation of sleep onset, without an associated increase in liver toxicity when compared to control. This suggests that the extract may not act by stimulating MDME but as a CNS stimulant (by enhancing excitatory neurotransmission, depressing inhibitory neurotransmission and/or altering presynaptic control of neurotransmitter release) to reduce the duration of pentobarbitone-induced sleep. Administration of the extracts after hepatotoxin challenge showed significant ($P<0.05$) reduction in PST, indicating an improvement in metabolic functions of the liver.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

Several phytochemical constituents such as flavonoids (Mangathayaru *et al.*, 2005), triterpenes (Marzouk, 2009), saponins (Tran *et al.*, 2001), tannins (Faure *et al.*, 1990) and alkaloids (Vijayan *et al.*, 2003) have been reported to possess hepatoprotective properties. The presence of such constituents in the methanolic extracts of *Balanites aegyptiaca, Boswellia dalzielii* and *Ipomoea asarifolia,* may be responsible for their observed antihepatotoxic properties.

Prophylactic administration of the individual extracts provided some degree of hepatoprotection against CCl_4-induced damage, better than that conferred by the standard drug, silymarin, while curative treatment revealed an almost total reversal of CCl_4 damage to normal, comparable to that of silymarin, indicating potent antihepatotoxic effects. Prophylactic and curative administration of the mixture of the three extracts offered no significant improvement in hepatoprotection or antihepatotoxicity when compared to the individual extracts.

Hepatoprotective properties of the extracts might be due to cell membrane stabilization, prevention of biliary dysfunction, antoxidant activity and/or inhibition of liver microsomal drug metabolizing enzymes. Antihepatotoxic properties could be attributed to one or more of the following mechanisms; repair of damaged hepatic tissue, antioxidant activity, reversal of biliary dysfunction and stimulation of protein synthesis. These results strongly suggest that *B. aegyptiaca, B. dalzielii* and *I. asarifolia,* may be therapeutically useful in the prevention and treatment of liver diseases.

Fractionation and further analysis need to be carried out to:

i) Isolate, purify and characterise the active principles in the extracts

ii) Establish the exact mechanism of hepatoprotective action of the extracts and the isolated active principles

iii) Analyze the dose-response relationships between the extracts and hepatic health

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