

**EVALUATION OF HEPATOPROTECTIVE EFFECT OF AQUEOUS EXTRACT OF BITTER KOLA (*Garcinia kola*)
SEEDS ON CARBON TETRACHLORIDE (CCL₄) INDUCED LIVER DAMAGE IN ADULT WISTAR RAT.**

BY

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**A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE
IN HUMAN ANATOMY**

**DEPARTMENT OF HUMAN ANATOMY
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AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

MARCH, 2014.

DECLARATION

I, Dauda Zainab, declare that work in the thesis entitled **“Evaluation of Hepatoprotective Effect of Aqueous Extract of Bitter Kola (*Garcinia Kola*) Seeds on Carbon Tetrachloride (CCl₄) induced Liver Damage in Adult wistar rats”** has been performed by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria under the supervision of Prof J.O. Hambolu and Dr. W.O Hamman.

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

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CERTIFICATION

This thesis entitled: “**Evaluation of hepatoprotective effect of aqueous extract of Bitter kola (*Garcinia kola*) seeds on carbon tetrachloride (CCl₄) induced liver damage in adult wistar rats**” by Dauda, Zainab meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University Zaria, for its contribution to scientific knowledge and literary presentation.

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This work is dedicated to ALMIGHTY ALLAH who is the “Source and Giver of all knowledge” and to the memories of my late father and son.

ACKNOWLEDGMENT

So many individuals have contributed so much to this project that it is obviously impossible to thank them satisfactorily. In the first place, I must register my heartfelt thanks to Almighty Allah for his guidance and protection throughout my period of study and for giving me the insight to write this thesis.

My profound gratitude goes to my supervisors, Prof J.O. Hambolu and Dr. W.O. Hamman for their tireless efforts and series of corrections which made this work complete. Thank you for all your efforts and encouragement which can only be paid by God alone who knows how best to reward you.

I want to use this opportunity to thank my family, for all their moral and financial support through my academics.

My special thanks to my mum Mrs. A.O. Dauda Sanni for her endless prayers and assistance throughout the course of my studies.

I also want to use this opportunity to thank my husband, for all his support through my academics. God bless you. I love you.

To my guardian, Mallam Saidu Sadiq of Faculty of Medicine, Department of Community Medicine, ABA, Zaria, thank you. May Allah continue to bless you and your family.

My special thanks also go to my Aunties, Hajiya Haleema Mohammed and Mrs. Mariam Mohammed for their endless prayers and assistances throughout the course of my studies.

My sincere gratitude and thanks to all my lecturers in the Department of Human Anatomy for their encouragements and support. They include Dr. B. Danborn, Dr. S. S.

Adebisi, Dr. A. O. Ibegbu, Dr. W. O. Hamman, Dr. Mrs. J. N. Alawa, Mr. D T. Ikymbe, Dr. A. U. Umana, Dr. A. Dahiru, Hajia Z. M. Bauchi, Mr. S. A. Musa, Mr. I. A. Iliya, Mr. A. A. Buraimo, Dr. A. A. Lawal and Mr. M. Tanko. Thank you all in a special way and God Bless you. To the technical staff particularly Mr. Peter Akpulu, in the Department of Human Anatomy, who contributed in the preparation of tissue sections, I say thank you and God Bless you.

My special thanks go to Mr. Yaro, Chief technician, Department of Pathology, ABUTH-Zaria, who took his time to process my tissues and the special stains. Thank you, God Bless you and your family.

My special thanks go to Dr. Sambo, Pathologist, Department of Veterinary Pathology, Faculty of Veterinary Medicine, ABU, Zaria, for offering useful advice in terms of observing and interpreting my micrographs.

This acknowledgement will be incomplete if I do not mention some of my colleagues and course mates namely, Abel Agbon, Jibrin Danladi Attai, Jibrin Murtala, Abdulsalam Ali, Abdullahi Jabata, Zubairu Rayyan, Elvis Godam Tams, Chibuke Onyesoh, Dr. Anyawu Lofty-John, Rukaya Salawudeen, Elizabeth, Shakira, Sabdat, Monsurat Gbadamosi, Hadeeza Rilwan, Vera Afuain and Progress Victor.

I want to thank my little baby, my son Mubarak Abdulmalik, for his understanding and care during my studying period. God bless you dear, I love you.

I sincerely appreciate the contributions of all whom for oversight, could not be mentioned, God will bless them for being so good and generous.

.

ABSTRACT

The aim of the study was to evaluate the hepatoprotective effect of aqueous extract of bitter kola (*Garcinia kola*) seeds on carbon tetrachloride (CCl₄) induced liver damage in adult wistar rats. The experimental animals (36 male and female) weighing between 150-250g were randomly divided into six groups. Each group comprised of 6 rats and was labeled as groups I, II, III, IV, V and VI. Group I (control) animals were administered distilled water orally daily for 2 weeks (volume per body weight) while group II (CCl₄ control) animals were administered distilled water orally daily for 2 weeks (volume per body weight) and carbon tetrachloride (CCl₄) 0.4 ml/kg intraperitoneally as a single application. Group III rats were administered 100 mg of silymarin / kg body weight once daily for 2 weeks followed by a single dose of CCl₄ (0.4 ml) on day 14 of the experiment. Group IV rats were administered 800 mg aqueous extract bitter kola (*Garcinia kola*) / kg body weight orally once daily for 2 weeks. Group V and VI rats were administered 800 mg and 400 mg aqueous extract bitter kola (*Garcinia kola* seed) orally once daily for 2 weeks followed by a single dose of CCl₄ (0.4 ml) on day 14 respectively. At the end of the experiment, blood samples were collected for serum analysis of levels of alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), alkaline phosphatase (**ALP**), superoxide dismutase (**SOD**), reduced glutathione (**GSH**) and catalase (**CAT**). Hepatic tissues were also collected for histopathological assessment of liver damage. Results obtained showed that the CCl₄ treated group caused significant increase in the levels of liver enzymes (AST, ALP and ALT). Bitter kola (*Garcinia kola*) seed aqueous extract caused significant decrease in the aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels in the serum of the extract treated groups. Histopathological and histochemical examinations of liver sections revealed distortion of histoarchitecture of the liver tissue, such as, sinusoidal congestion, necrosis, steatosis and fibrosis was observed in group II. The administration of aqueous extract of bitter kola (*Garcinia kola*) seed remarkably inhibited histoarchitectural distortion induced by CCl₄ administration. Hepatoprotective activity of the extract at dose of 400mg/kg was

comparable to the reference drug. *Garcinia kola* aqueous seed extract showed a remarkable hepatoprotective and antioxidant activity against CCl₄-induced hepatotoxicity as observed from the serum marker enzymes and antioxidant levels in liver tissues. CCl₄-induced a significant rise in AST, ALT, ALP with a reduction of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). Treatment of the rats with the extract significantly ($p < 0.05$) altered serum marker enzymes and antioxidant levels to near normal compared with CCl₄-treated rats (group II). The activity of the extract at dose of 400mg/kg (group VI) was comparable to the standard drug confirmed by histopathological examinations of liver sections. Results indicated that *Garcinia kola* aqueous seed extract has hepatoprotective and antioxidant properties against CCl₄-induced hepatotoxicity in Wistar rats.

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ABBREVIATIONS

World health Organisation (WHO)

Carbon tetrachloride (CCL₄)

Hepatitis B (HBV)

Low-density lipoproteins (LDL)

Benzophenone, Garcinia biflavinones (GB-1, GB-2)

50% lethal dose (LD50)

Ribonucleic acid (RNA)

Adenosine diphosphate (ADP)

Oxidized reduced glutathione (GSSH)

Intraperitoneal (IP)

Alanine aminotransferase (ALT)

Aspartate aminotransferase (AST)

Alkaline phosphatase (ALP)

Superoxide dismutase (SOD)

Reduced glutathione (GSH)

Catalase (CAT)

Reagent 1 (R1)

Reagent 2 (R2)

Hydrogen peroxide (H₂O₂)

Millimole per liter (mmol/l)

Microlitre (μl)

Milligram (mg)

Nanometer (nm)

Absorbance (A)

A_1 (Initial absorbance)

A_5 (Final absorbance)

Molar extinction coefficient (ϵ)

Concentration (C)

Path length of the cuvette (l)

Gordon and Sweets (G and S)

Statistical Package for Social Scientist (SPSS)

Standard mean of error (SEM)

Analysis of variance (ANOVA)

Aqueous extract of *Garcinia kola* (AEGK)

Haematoxylin and Eosin (H and E)

Water (H_2O)

Hydrogen peroxide (H_2O_2)

Oxygen (O_2)

Silymarin (Sil)

Percentage (%)

CHAPTER ONE

1.0 Introduction

Plants have been an important source of medicine for thousands of years. Today, the World Health Organisation (WHO, 2000) estimated that 80% of people still rely on traditional remedies such as herb for their medicines (Tripathi and Tripathi, 2003). Plants are also sources of many modern medicines. It is estimated that approximately a quarter of processed drugs contain plant extracts or active ingredient obtained from or modelled on plant substances (Tripathi and Tripathi, 2003).

Garcinia kola belongs to the family Guittiferae, and it is commonly referred to as 'Bitter Kola'. The plant has the popular acronym "wonder plant" amongst the South-Western Nigerian people because every part of it have been found to be of medicinal importance (Olaleye *et al.*, 2000). In Nigeria, it is referred to as Namijin-goro in Hausa, Orogbo in yoruba, Akiilu in Igbo and Oro in Epira languages. *Garcinia. kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhoea, laryngitis, and bronchitis (Iwu, 1993; Adesina *et al.*, 1995).

The seed is masticatory and is used to prevent chest colds, and cough and can be used as well to treat headache (Ayensu, 1978). Iwu (1993) reported the use of this plant for the treatment of jaundice, high fever, purgative and chewing stick. The plant has also been found useful in the treatment of stomach ache and gastritis (Ajebesone and Aina, 2004).

Liver diseases are some of the fatal diseases in the world today, posing a serious challenge to the international public health (Ahsan *et al.*, 2009). Increase in the incidence of alcoholism, substance abuse (toxic chemicals) and other unhealthy life style such as eating fatty foods have contributed to the morbidity and mortality due to liver diseases (Franchesca *et al.*, 2010). Some

of the commonly known disorders of the liver include viral hepatitis, alcoholic liver diseases, non-alcoholic liver diseases, auto-immune liver diseases, metabolic liver disease, drug-induced liver injury, gall stones, etc. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Dianzani *et al.*, 1991).

Carbon tetrachloride (CCL₄) has been used extensively to study liver injury induced by free radicals in an animal model system. CCL₄ -treated rats are widely used to study liver damage where it was reported that CCL₄ induced not only necrosis but also apoptosis in rat liver (Shi *et al.*, 1998; Sun *et al.*, 2001). Although the mechanism by which CCL₄ causes liver damage is unclear, several lines of evidence suggest that the liver damage could be caused by free radical metabolites (Williams *et al.*, 1990). CCL₄ is converted to the trichloromethyl radical by cytochrome *P*-450 through a 1-electron reduction. A fatty acid radical is generated by the reaction between trichloromethyl radical and unsaturated fatty acids, and lipid peroxidation follows, Shi *et al.*, 1998; Sun *et al.*, 2001).

1.1 Statement of the Research Problem

In recent times, hepatotoxicity and liver related diseases are becoming prevalent especially in developing countries, such as, Nigeria. Some of these diseases result from the kind of diet and life style of the individuals, (Franchesca *et al.*, 2010).

Over the years, the crude extracts of *Garcinia kola* seeds have been used traditionally in the management of ailments especially liver disorders, (Iwu, 1990). Clinical investigations using *Garcinia kola* are few and a clear understanding of how it exerts its effect is unknown.

1.2 Aim of the study

To evaluate the hepatoprotective effect of aqueous extract of bitter (*Garcinia kola*) seeds on CCL₄-induced hepatic toxicity.

1.3 Objectives of the study

- To evaluate the hepatoprotective effect of aqueous extract of bitter kola seeds on CCL₄-induced hepatic toxicity using established hepatoprotective drug, silymarin as a standard.
- To investigate the histological changes of the liver resulting from oral consumption of *Garcinia kola* extract using H & E stain.
- To investigate the activities liver enzymes (ALT, AST, ALP, SOD, GSH and CAT) using biochemical techniques.

1.4 Justification of the study

Popular and indigenous medical systems, often, are the only source of immediate help for populations that lack access to formal health resources. Because plants are integral elements of these medical systems, it is important to understand indigenous therapeutic objectives in using plant medicine.

The improvement of health in developing nations requires an understanding of indigenous and popular concepts of liver related diseases, including its etiology and treatment.

1.5 Hypothesis

Oral administration of aqueous extract of bitter kola (*Garcinia kola*) seeds has protective effect on CCl₄ induced liver toxicity in adult wistar rats.

1.6 Significance of the study

The study is significant as it may lead to the design of plant based remedy for hepatoprotection that are natural, easily accessible, cheap with minimal side effects when compared to the convectional hepatoprotective drugs e.g erythromycin, alphaamethyldopa (Shah, 2010).

Results from this study may be important as it may enlighten the public on the effect of ingesting *G.kola* particularly because it is an inexpensive and effective prophylactic and/or treatment against liver toxicity/ diseases

CHAPTER TWO

2.0 Literature Review

2.1 The Liver

The liver is the largest organ in the body. It weighs approximately 1500g and accounts for one-fortieth of the adult body weight. It lies on the right and left upper quadrants inferior to the diaphragm which separates it from the pleura, lungs, pericardium and heart (Dalley and Moore, 1999). The liver is divided into a larger right and a smaller left lobe, separated superiorly by the falciform ligament and posterior-inferior by an H-shaped arrangement of fossae` (Ellis, 2006).

2.1.1 Functional Parts of the Liver

The liver has functionally independent right and left parts that are approximately equal in size (Dalley and Moore, 1999). Each part has its own blood supply from the hepatic artery and portal vein, and its own venous and biliary drainage. On the visceral surface, the right part of the liver is demarcated from the left by the gall bladder fossa inferiorly and the fossa from the inferior vena cava superiorly (Dalley and Moore, 1999; Ellis, 2006). An imaginary line over the diaphragmatic surface of the liver that runs from the fundus of the gall bladder to the inferior vena cava separates the two parts which includes the caudate and quadrate lobes (Dalley and Moore, 1999).

2.1.2 Porta Hepatis

The porta hepatis is a transverse fissure on the visceral surface of the liver between the caudate and quadrate lobes, where the portal vein and the hepatic artery enter the liver and the hepatic ducts leave. The porta hepatis gives passage to the portal vein, hepatic artery, hepatic nerve plexus, hepatic ducts and lymphatic vessels (Dalley and Moore, 1999).

2.1.3 Vessels and Nerves of the Liver

The liver receives blood from two sources i.e. the portal vein (70%) and the hepatic artery (30%) (Dalley and Moore, 1999). The portal vein is a short wide vein formed by the superior mesenteric and splenic veins posterior to the neck of the pancreas and ascends anteriorly to the inferior vena cava, and divides at right end of porta hepatis into right and left branches that ramify within the liver (Dalley and Moore, 1999). The hepatic artery is a branch of the celiac trunk and may be divided into the common hepatic and the hepatic artery proper (Dalley and Moore, 1999).

The hepatic artery carries well-oxygenated blood from the aorta and the portal vein carries poorly oxygenated but nutrient filled blood from the gastrointestinal tract (except the inferior part of the anal canal) to sinusoids of the liver. At or close to the porta hepatis, the hepatic artery and portal vein terminate by dividing into the right and left branches which supply the right and left parts of the liver respectively (Dalley and Moore, 1999).

2.1.4 Nerve Supply of the Liver

The nerves of the liver are derived from the nerve plexus, which is the largest derivative of the celiac plexus. The hepatic plexus accompanies the branches of the hepatic artery and portal vein to the liver (Dalley and Moore, 1999). It consists of fibres from the anterior and posterior vagal trunks. Nerve vessels accompany the vessels and bile ducts of the portal triad within the liver (Dalley and Moore, 1999).

2.1.5 Lymphatic Drainage

The liver is a major lymph-producing organ. It produces between one-quarter and one-half of the lymph received by the thoracic duct. The lymphatic vessels of the liver occur as superficial lymphatics in the subperitoneal fibrous capsule of the liver and as deep lymphatics in the connective tissue of that accompany the ramification of the portal triad and hepatic veins (Dalley and Moore, 1999). The lymphatics on the anterior aspects of the diaphragm and visceral surfaces of the liver and those accompanying the portal triad drain into the hepatic lymph nodes. Efferent nodes drain into celiac lymph nodes and the chyle cistern (Dalley and Moore, 1999). The lymphatics on the posterior aspects of the diaphragmatic and visceral surface into the phrenic lymph nodes.

2.1.6 Histology of the Liver.

Sections through the liver show polygonal areas that constitute the hepatic lobules. In some species, the lobules are distinctly demarcated by connective tissue septa, but in the human liver, the connective tissue is scanty and lobules often appear to merge with one another. In transverse sections, each lobule appears to be made up of cords of liver cells that are separated by sinusoids (Singh, 2002). The liver cells are arranged in the form of plates that branch and anastomose with one another to form a network and the spaces within the network are occupied by sinusoids (Singh, 2002). Along the periphery of each lobule, there are angular intervals filled by connective tissue and these intervals are called portal canals. Each canal forms a connective tissue network permeating the entire liver substance (Singh, 2002). Each canal contains an interlobular branch of the portal vein, an interlobular terminal, a branch of the hepatic artery and an interlobular bile duct. These three structures collectively form the portal triad (Singh, 2002). Blood from the hepatic artery enters the sinusoids at the periphery of the lobule and passes towards its centre. The sinusoids open into a central vein which occupies the centre of the lobule (Singh, 2002).

The vessels in a portal triad usually give branches to parts of three adjoining lobules. The area of the liver tissue (i.e. comprising of three hepatic lobules) supplied by one branch of the portal vein is regarded to as the portal lobule (Singh, 2002). On the other hand, a portal acinus consists of the area of the liver tissue supplied by one hepatic arteriole running along the line of junction of two hepatic lobules. Two central veins lie at the acinus (Singh, 2002).

The liver is covered by a connective tissue capsule (Glisson's capsules), which extends into the liver substance through the portal canals where it surrounds the portal triads. Sinusoids are surrounded by rectangular fibres while the connective tissues do not intervene between adjoining liver cells (Singh, 2002).

Kupffer cells are hepatic macrophages derived from circulating blood monocytes. The cells are long-term hepatic residents, lying within the sinusoidal lumen, attached to the endothelial surface. These cells originated in the bone marrow, and form a major part of the mononuclear phagocyte system responsible for removing cellular and microbial debris from the circulation, and secreting cytokines involved in defence. Kupffer cells remove aged and damaged red cells from the hepatic circulation, a function normally shared with the spleen, but fulfilled entirely by the liver after splenectomy. Kupffer cells are irregular in shape, with long processes extending into the sinusoidal lumen (Standrig, 2006).

2.1.7 Functions of the Liver

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

- **Fat metabolism**
 - Oxidising triglycerides to produce energy.

- Synthesis of plasma lipoproteins.
- Synthesis of cholesterol and phospholipid.
- **Carbohydrate metabolism**
 - Converting carbohydrates and proteins into fatty acids and triglyceride.
 - Regulation of blood glucose concentration by glycogenesis, glycogenolysis and gluconeogenesis.
- **Protein metabolism**
 - Synthesis of the plasma proteins, including albumin and clotting factors.
 - Synthesis of the non-essential amino acids.
 - Detoxification of metabolic waste products, e.g. deamination of amino acids and production of urea.
- **Storage**
 - Storage of glycogen, vitamins, iron.
- **Intermediary metabolism**
 - Detoxification of various drugs and toxins, such as alcohol.
- **Secretion**
 - Synthesis and secretion of bile; bile contains many of the products of the above processes. (Young *et al*, 2007).

According to Singh (2002), the functions of the liver include:

- a) The liver acts as an exocrine gland for the secretion of bile
- b) It plays a prominent role in the metabolism of carbohydrate, proteins and fats
- c) It acts as a store for various substances including glucose (glycogen), lipids, vitamins and iron
- d) It plays a protective role by detoxifying substances including drugs and alcohol

e) During fetal life, the liver serves as Centre for hematopoiesis.

2.1.8 Developmental Stages of Liver

The liver develops from an endodermal bud that arises from the ventral aspect of the gut, at the junction between foregut and midgut. This bud grows into the ventral mesogastrium and passes through it to the septum transversum. It enlarges and soon shows a division into a large cranial part called the pars hepatica, and a smaller caudal portion called the pars cystica. The pars hepatica divides into right and left parts, each of which forms one lobe of the liver. The left and right divisions of the pars hepatica enlarge and extend into the septum transversum, the cells arising from them are broken up into interlacing columns called hepatic trabeculae. The umbilical and vitelline veins which lie in the septum transversum are broken up to form the sinusoids of the liver. The sinusoids are also formed from the mesenchyme of the septum transversum. The endodermal cells of the hepatic bud give rise to the parenchyma of the liver and the bile capillaries. The mesoderm of the septum transversum forms the capsule and fibrous tissue of the liver (Singh, 2002).

2.1.9 Liver Diseases

Liver disease is a term used for a collection of conditions, diseases and infections that affect the cells, tissues, structures or functions of the liver. It is also called hepatic disease (Shah, 2010). Most of the hepatotoxic chemicals damage the liver mainly by inducing lipid peroxidation and other oxidative damages (Valarmathi *et al.*, 2010).

Hepatotoxicity is one of the common ailments resulting into serious debilitating effects ranging from severe metabolic disorders to even mortality (Kumar *et al.*; 2009). This hepatotoxicity in most cases is due to free radicals which are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism (Tiwari, 2009).

Liver diseases are some of the fatal diseases in the world today, posing a serious challenge to international public health (Ahsan *et al.*; 2009). The increase in the incidence of alcoholism, cigarette smoking, substance abuse and other unhealthy lifestyle options like eating fatty foods have contributed to the morbidity and mortality due to liver diseases (Franchesca *et al.*; 2010). Some of the commonly known disorders of the liver include viral hepatitis, alcoholic liver disease, non-alcoholic liver disease, auto-immune liver disease, metabolic liver disease, drug-induced liver injury, gall stones etc.

Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug related agencies. Drug induced liver toxicity is a common cause of liver insufficiency or failure. It accounts for approximately half of the cases of acute liver failure and mimics all forms of acute and chronic liver diseases .Some drugs that have hepatotoxic effect include acetaminophen (paracetamol), chloroquine, isoniazid carbon tetrachloride ,ibuprofene and ethanol (Mahalakshmi, *et al.*; 2010).

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages (Negi *et al.*, 2008). According to WHO, estimates show that, globally 170 million people are chronically infected with hepatitis C alone and every year, 3-4 million are newly added to the list. Also, there are more than 2 billion infected by hepatitis B (HBV) and over 5million are getting infected with acute HBV annually (WHO, 2000; Negi *et al.*, 2008). At present, there are nearly 5.5 million chronic liver disease patients in USA alone with more than 5000 liver transplants performed in adults and more than 500 performed in children every year (NIDDK/NIH; Negi *et al.*, 2008).

2.2. Medicinal Plants

Nature is and will still serve as the man's primary source for the cure of his ailments. However, the potential of higher plants as sources for new drugs is still largely unexplored. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.

In all countries of the world there exists traditional knowledge related to the health of humans and animals. The importance of traditional medicine as a source of primary health care was first officially recognised by the World Health Organisation (WHO) in the Primary Health Care Declaration of Alma Ata (1978) and has been globally addressed since 1976 by the Traditional Medicine Programme of the WHO. That Programme defined traditional medicine as: "the sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing." In Africa, traditional healers and remedies made from plants play an important role in the health of millions of people.

Historically, plants have been used in folk medicine to treat various diseases and these have been shown to be rich in natural antioxidants (Valarmathi *et al.*, 2010). Many researchers have examined the effect of plants used traditionally by indigenous people to support her function and treat diseases such as diseases of the liver. In most cases, research has confirmed traditional experience by discovering the mechanism and mode of action of these plants (Valarmath *et al.*, 2010).

In spite of tremendous advances in modern medicine, there are no effective drugs available to stimulate liver function, offer protection to the liver from damage or help regenerate hepatic cells (Shanmuga & Venkataraman, 2006). Plant based formulations are frequently employed for liver diseases, but there are a few effective suitable drugs available (Chatterjee, 2000a; Muragesh *et al.*, 2005).

2.2.1 Hepatoprotective Plants

Liver disease is still a worldwide health problem (Kumar *et al.*, 2010). Unfortunately, conventional or synthetic drugs in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Kumar *et al.*, 2010). In view of severe undesirable side effects of synthetic agents, there is a growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity (Chatterjee, 2000b). Clinical research in this century has confirmed the efficacy of several plants in the treatment of liver disease and, basic scientific research has uncovered the mechanisms by which some plants afford their therapeutic effects (Mahalakshmi *et al.*, 2010). Valarmathi *et al.*, (2010) in their study demonstrated that the alcoholic extract of *Mollugo pentaphylla* exhibited significant protection from carbon tetrachloride induced liver damage in experimental model. They suggested that the mechanism of action of this plant may be due to its antioxidant and free radicals scavenging properties.

Study by Jain *et al.*, (2009) showed that both aqueous and ethanolic extracts of *Amorphophallus campanulatus* (Robx) have potent hepatoprotective action against carbon tetrachloride-induced hepatic damage in rats though the ethanolic extract was found to be more potent than the aqueous extract. They suggested that the possible mechanism through which it

mediates its effect may be due to its free radical scavenging properties which may be as a result of the presence of flavonoids in the extracts.

Other studies showed that the methanolic extract of *Sphaeranthus indicus* exhibited hepatoprotective effect against acetaminophen-induced hepatotoxicity in rats (Tiwari and Khosa, 2009). Aqueous extract of *Angelica glauca edgew* root has also been reported to have hepatoprotective effect against carbon tetrachloride induced hepatotoxicity in rats and its mechanism of action was suggested to be due to the inhibition of Cytochrome P-450-dependent oxygenase activity (Joshi *et al.*, 2008).

Das *et al.*, (2009) demonstrated that ethanolic extract of *Moringa pterygosperma* possessed a dose dependent hepatoprotective activity in rats, showing a higher potency at a high dose of 250 mg/kg as compared to 150mg/kg..

Franchester *et al.*, (2010) demonstrated the hepatoprotective effect of *Citrus microcarpa* Bunge (family-Rutaceae) fruit peel extract against acetaminophen-induced hepatotoxicity in male SD rats. This hepatoprotective effect was found to be comparable to that of Silymarin though its mechanism of action is not known. Muragesh *et al.*, (2005). In their study found that *Berberistinctoria* Lesch leaves had hepatoprotective effect against paracetamol-induced hepatotoxicity in rats suggesting that its mechanism of action to be due to its antioxidant properties.

Furthermore, Asha (2001) demonstrated that a suspension of *Marmodica subangulata* leaf had a dose dependent ability to protect rats from paracetamol-induced hepatotoxicity i.e. the extract

was found to be more effective at a low dose of 50mg/kg compared to a higher dose of 500mg/kg. Its mechanism of action is unknown.

Vitexnegundo Linn.(Verbanaccae) leaves have been found to possess hepatoprotective activity against ibuprofene-induced hepatotoxicity in rats though its mechanism of action is not known (Mahalakshmi *et al.*, 2010) while bergamot orange was found to have a weak hepatoprotective effect against carbon tetrachloride induced acute liver toxicity (Karace *et al.*, 2005). Besides, *Sclerocaryabirrea* aqueous stem-bark extract was also found to ameliorate ethanol-carbon tetrachloride induced liver damage in rats (Garba *et al.*, 2006).

2.3. The Plant, *Garcinia kola*.

Garcinia kola Heckel (Guttiferae) is a large fruit tree that abounds in the rain forest belt of Southern Nigeria. The seed (“bitter kola”) is used in Nigerian herbal medicine to treat diarrhoea, hepatitis, asthma, dysmenorrhoea or menstrual cramps (Dalziel, 1937). The alkaloid and biflavonoid extracts of *G. kola* seed exhibited the following effects: dose–dependent spasmolytic effects on uterine and gastrointestinal smooth muscle (Braide, 1991); deterioration of reproductive function (Braide *et al*, 2003); anti-inflammatory and antipyretic effects, antihepatotoxic effect (Akintonwa and Essien, 1990; Braide, 1991; Iwu *et al*, 1987); and antidiabetic activity (Iwu *et al*, 1990). Toxicological studies on chronic ingestion of *G. kola* seed was observed to induce histopathological changes in liver parenchymal cells, renal tubular epithelium and duodenal villous epithelium (Braide and Grill, 1990). The ingestion of *G. kola* seed also caused mild bronchodilatation in man (Orie and Ekon, 1993).

Other effects of *G. kola* seed extracts include protection against carbon tetrachloride induced erythrocyte damage (Adaramoye and Akinloye, 2000) and inhibitory effect on lipid

peroxidation (Esimore *et al*, 2005; Farombi *et al*, 2000). These spectra of effects, directly or indirectly is involved at the biochemical level with electrolytes and trace metals, (Agada and Braide, 2009). For example, the spasmolytic effect of *G. kola* seed on smooth muscle could be due to inhibition of transmembrane influx of calcium ions into the cytosol. Several lines of evidence indicate that the development of atherosclerosis is related to free-radical activity, lipid peroxidation and oxidative modifications of low-density lipoproteins (LDL). Natural antioxidants such as the enzyme copper, zinc superoxide dismutase (Cu, Zn SOD) abound. Since zinc is an essential component of Cu, Zn SOD, any deficiency of zinc could induce an increase in tissue oxidative damage (Socha, 1992). *Garcinia kola* seed has been shown to exert antioxidant activity (Adaramoye *et al*, 2005) and this observation may not be unconnected with alterations in the levels of zinc in serum, and hence the body.

2.3.1 Medicinal properties and Uses of *Garcinia kola*

Garcinia kola is regarded as a wonder plant because every part of the plant (bark, leaves, root and wood) has been found to be of medicinal importance. The medicinal importance of bitter kola is based mainly on the phytochemical components of the plant. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves or other parts of plants that interplay with nutrients and dietary fibre to protect them. Recent research demonstrates that they can protect humans against diseases as well as, in risk reduction for a variety of chronic or inflammatory conditions. Some of the well-known phytochemicals include; lycopenes found in tomatoes, isoflavones in soy and flavonoids in fruits. Group of polyphenolic compounds in the human diet and are found ubiquitously in plants. There are different types of flavonoids and each appears to have protective effects including anti-inflammatory, anti-oxidant, antiviral, and anti-carcinogenic properties. From its roots to its leaves, the plant is known to contain several phytochemicals noted for their medicinal importance (Iwu *et al.*, 1990).

Some of the phytochemical compounds that have been isolated from *Garcinia kola* includes; oleoresin, class, tannin, saponins, alkaloids, and cardiac glycoside,

Others are biflavonoids such as kola flavonone, and 2 hydroxy flavonoids. In addition, two new chromanols; garioic and garcinal together with - tocotrienol have been reportedly isolated from bitter kola (Terashima *et al.*, 2002).

The biflavonoids are the more abundant compounds in *Garcinia kola*, while the kolaflavones are the major components of kolavirons. Other constituents of *G. kola* include benzophenone, *Garcinia* biflavinones (GB-1, GB-2) and kola flavonone.

2.3.2 Physiological Properties of *Garcinia kola* seeds

G. kola seed is believed to contain a wide spectrum of organic compounds such as flavonoids which confer on it some antimicrobial and antifungal actions against gram negative and gram positive microorganisms (Akpanta *et al.*; 2005). The biological activities of flavonoids include action against allergies, inflammation, free radicals, and hepatotoxins (Terashima *et al.*, 2002). *G. kola* seed is also used in the treatment of liver disease and diarrhoea (Iwu *et al.*, 1990; Braide, 1991). It is also reported to be useful in the treatment of diabetes, bronchitis and throat infections. Traditionally the plant is used as a natural antimicrobial.

Other medicinal properties of the plant include its usage in the traditional treatment of skin infections in Liberia and Congo Democratic Republic. The powdered bark of the plant is applied to malignant tumors, cancers etc. In Congo, a bark decoction is taken for female sterility and to ease child birth, the intake being daily till conception is certain and then at half quantity throughout the term. The bark is used as a strong anti-diuretic in the treatment of urinary decongestion and chronic urethral discharge.

In Ivory Coast, a decoction of the bark is taken to induce the expulsion of a dead fetus, while the seed and the bark are taken for stomach pain. In Sierra Leone, the roots and bark are taken as a tonic for sexual dysfunction in men. The bark is also added into palm wine to improve its potency. In Nigeria, a cold water extract of the roots and bark with salt are administered to cases of *Ukwala* (bronchial asthma or cough) or *agbo* (vomiting) (Iwu *et al.*, 1990).

The medicinal properties of bitter kola can be classified under:

- Purgative
- Antiparasitic
- Antimicrobial

The antimicrobial nature of this plant has been attributed to the benzophenone and flavonones present in the plant while the anti-inflammatory effects of *G. kola* is believed to result from the inhibition of the cyclooxygenase enzyme. Flavonoids are generally found in a variety of foods, such as oranges, tangerines, berries, apples and onions.

2.3.4 Functions of phytochemicals

Most phytochemicals are known to possess many properties which make them vital to both plants and animals. Some of these properties are:

- **Antioxidants:** most phytochemicals have antioxidant activity and protect cells against oxidative damage and reduce the risk of developing certain types of cancer. Some phytochemicals with antioxidant activities include:
 - a. carotenoids (found in fruits)
 - b. flavonoids (found in fruits, vegetables)

c. polyphenols (tea, grapes).

- **Anti-microbial properties:** The phytochemical allicin from garlic has anti-bacterial properties while benzophenone and flavanone found in bitter kola also possess anti-microbial properties
- **Physiological activities:** some phytochemicals bind physiologically to cell walls interfering with the ability of pathogens to bind to cell receptors.

2.3.5 Toxicology

Acute toxicity was determined in healthy adult albino mice of either sex as previously described (Asare *et al.*, 2011). Two groups of 5 mice/group received single oral dose of *G. kola* seed extract at 1200 and 2400 mg/kg body weight. The animals were observed continuously for 1 h, then hourly for the next 4 h, intermittently over the next 48 h and at least once a day for two weeks. Physical manifestations of toxicity such as writhing, gasping, salivation, hyperactivity, drowsiness and death were looked for during animal observations.

The studies carried out using oral doses of 1200 and 2400 mg/kg *G. kola* extract did not cause any obvious signs of toxicity as assessed by behavioural changes by the experimental rats. Rats looked active and healthy after two weeks of observation. This shows that the plant seeds are relatively safe for consumption.

2.4. Silymarin

More than 4000 different flavonoids are currently known; they are ubiquitous not only in the plant kingdom, where they are particularly abundant in the photosynthetic cells of higher plants, but also in the animal kingdom. For centuries they have been attributed numerous therapeutic properties and many have been used as popular therapeutic remedies. Compounds such as quercetin, taxifolin and silymarin have been used as active ingredients both alone and as components of complex chemical preparations. Silymarin is a flavonolignan that has been

introduced fairly recently as a hepatoprotective agent. It is the most well-known compound of the flavonoids, thanks to its well-defined therapeutic properties. It is extracted from the seeds and fruit of *Silybummarianum* (Composite) and in reality is a mixture of three structural components: silibinin, silydianine and silychristine. The structure of the constituents of silymarin (Figure 1) was clarified in the 1960s (Valenzuela and Garrido, 1994).

The main chemical difference between silymarin and other flavonoids is that its isomers are substituted by a coniferyl alcohol group. Of the three isomers that constitute silymarin, silibinin is the most active. From a medical point of view, silymarin and silibinin have been found to provide cytoprotection and, above all, hepatoprotection. Silymarin is used for the treatment of numerous liver disorders characterised by degenerative necrosis and functional impairment (Vogel *et al.*, 1975).

Furthermore, it is able to antagonise the toxin of *Amanita phalloides* (Vogel *et al.*, 1975) and provides hepatoprotection against poisoning by phalloidin, galactosamine, thioacetamide, halothane and carbon tetrachloride. The compound also protects hepatocytes from injury caused by ischaemia, radiation, iron overload and viral hepatitis (Luper, 1998). Silymarin is included in the pharmacopoeia of many countries under the trademark LegalonTM or HepatronTM and is often used as supportive therapy in food poisoning due to fungi and in chronic liver disorders, such as steatosis and alcohol-related liver disease (Ferenci *et al.*, 1989).

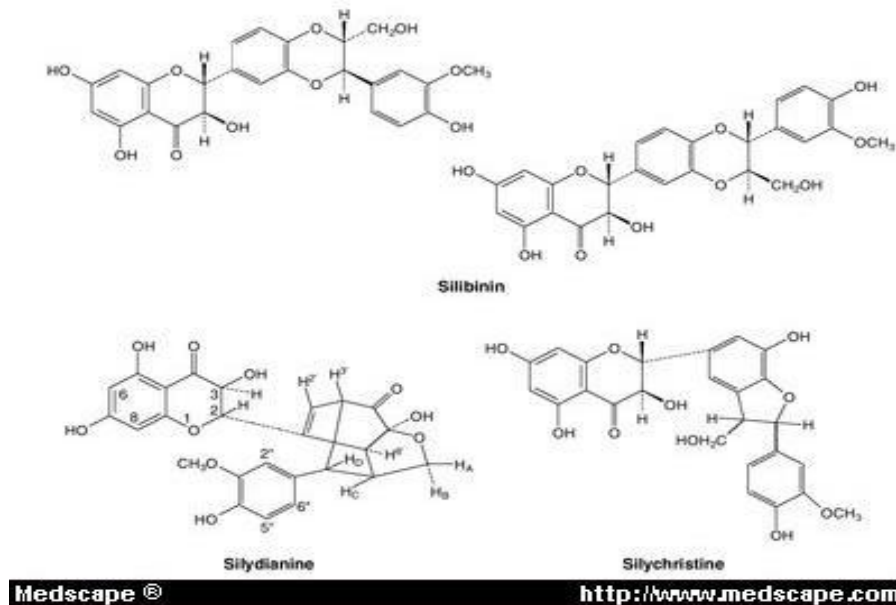


Figure 1: Chemical Structure of Silymarin

2.4.1 Antioxidant Properties of Silymarin

Flavonoids usually possess good antioxidant activity. The water-soluble dehydro succinate sodium salt of silibinin is a powerful inhibitor of the oxidation of linoleic acid-water emulsion catalysed by Fe^{2+} salts (Ferenci, 1989). It also inhibits in a concentration-dependent way the microsomal peroxidation produced by NADPH Fe^{2+} ADP, a well-known experimental system for the formation of hydroxy radicals (Valenzuela and Guerra, 1986). In studies performed in rat hepatic microsomes, it has been demonstrated that lipid peroxidation produced by Fe (III)/ascorbate is inhibited by silibinin dihemisuccinate; the inhibition is concentration-dependent. It has been shown that silymarin is as active as quercetin and dihydroquercetin, and more active than quercitrin, in terms of antiperoxidant activity, independent of the experimental model used to produce peroxidation (Ferenci, 1989).

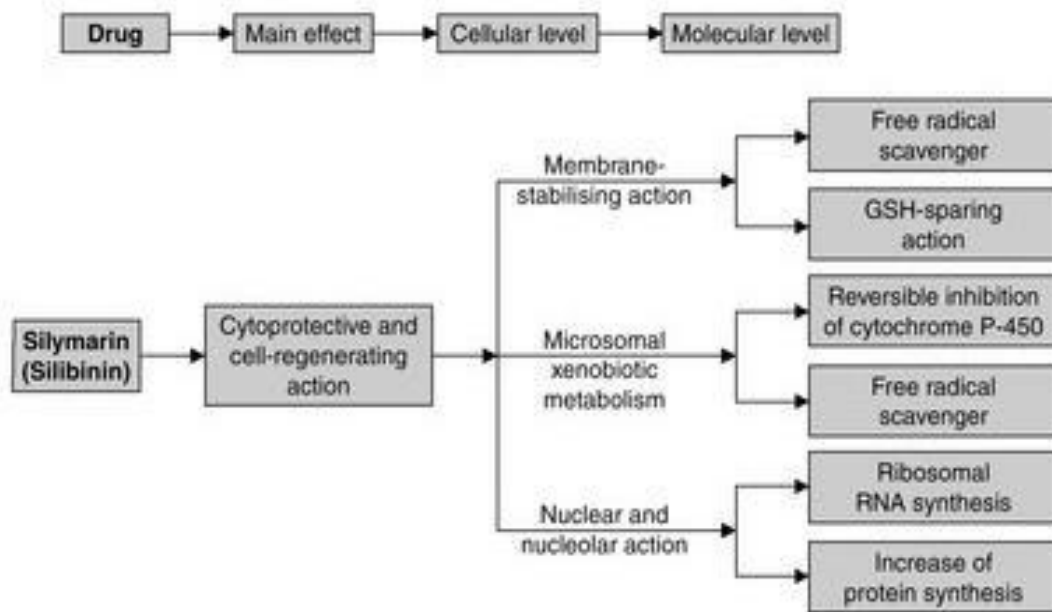
The hepatoprotection provided by silymarin appears to rest on four properties:

- activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of GSH;

- ability to regulate membrane permeability and to increase membrane stability in the presence of xenobiotic damage;
- capacity to regulate nuclear expression by means of a steroid-like effect;
- inhibition of the transformation of stellate hepatocytes into myofibroblasts, which are responsible for the deposition of collagen fibres leading to cirrhosis.

Silymarin and silibinin inhibit the absorption of toxins, such as phalloidin or a-amanitin, preventing them from binding to the cell surface and inhibiting membrane transport systems. Furthermore, silymarin and silibinin, by interacting with the lipid component of cell membranes, can influence their chemical and physical properties. Studies in erythrocytes, mast cells, leucocytes, macrophages and hepatocytes have shown that silymarin renders cell membranes more resistant to lesions (figure 2). (Valenzuela and Garrido, 1994)

Furthermore, the well documented scavenging activity of silymarin and silibinin can explain the protection afforded by these substances against hepatotoxic agents. Silymarin and silibinin may exert their action by acting as free radical scavengers and interrupting the lipid peroxidation processes involved in the hepatic injury produced by toxic agents. Silymarin and silibinin are probably able to antagonise the depletion of the two main detoxifying mechanisms, GSH and superoxide dismutase (SOD), by reducing the free radical load, increasing GSH levels and stimulating SOD activity. Furthermore, silibinin probably acts not only on the cell membrane, but also on the nucleus, where it appeared to increase ribosomal protein synthesis by stimulating RNA polymerase I and the transcription of rRNA. The stimulation of protein synthesis is an important step in the repair of hepatic injury and is essential for restoring structural proteins and enzymes damaged by hepatotoxins (Valenzuela and Garrido, 1994).



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Figure 2: Mechanism of Action of Silymarin as proposed by Valenzuela and Garrido (Valenzuela and Garrido, 1994)

2.4.3 Pharmacokinetics of Silymarin

Silymarin is not soluble in water and is usually administered in capsules as a standard extract (70 to 80% silymarin). Absorption after oral administration is rather low, with recovery in the bile in rats ranging from 2 to 3%. Peak plasma concentrations are achieved in 4 to 6 hours, both in animal and in humans. Silymarin is mainly excreted in the bile and, to a lesser degree, in the urine. Its elimination half-life ranges from 6 to 8 hours (Nevien, 2012).

2.4.4 Toxicity of Silymarin

The acute toxicity of silymarin has been studied in mice, rats, rabbits and dogs after intravenous infusion. The 50% lethal dose (LD50) values are 400 mg/kg in mice, 385 mg/kg in rats and 140 mg/kg in rabbits and dogs. However, these values are only approximate, as they

depend on the infusion rate. When the compound is given by slow infusion (over 2 to 3 hours), values of 2g/kg may be recorded in rats. After oral administration tolerance is even higher, with values over 10 g/kg. In the event of acute intoxication, the cause of death seems to be cardiovascular failure. Similar results have also been obtained by (Vogel *et al.*, 1975).

Other experiments to assess the acute toxicity of silymarin were performed in beagle dogs, rabbits, Wistar rats and NMRI mice after an intravenous bolus dose. Silymarin was used as the hemisuccinate sodium salt and the animals were kept under observation for 14 days. The LD50 was 1050 and 970 mg/kg in male and female mice, respectively, and 825 and 920 mg/kg in male and female rats, respectively. The mean lethal dose for rabbits and the maximum tolerated dose in dogs were calculated to be about 300 mg/kg (Vogel *et al.*, 1975).

These data demonstrate that the acute toxicity of silymarin is very low. Similarly, its sub-acute and chronic toxicity are very low; the compound is also devoid of embryotoxic potential.

2.4.5 Dosage and Administration of Silymarin

In the clinical trials described, the daily oral dose of silymarin used ranged from 280 to 800mg. This is equivalent to 400 to 1140mg of standardised extract containing 70% silymarin. The recommended dosage for active disease is 140mg of silymarin (200mg of extract) three times daily. If the preparation silipide (silymarin-phosphatidylcholine) is used, 100mg three times daily is the appropriate dosage (Flora *et al.*, 1998). At higher dosages (>1500 mg/day) silymarin may have a laxative effect due to an increase in secretion and bile flow (Luper, 1998). Moderate allergic reactions have also been reported (Luper, 1998).

2.5. Carbon Tetrachloride

Carbon tetrachloride may be found in both ambient outdoor and indoor air. The primary effects of carbon tetrachloride in humans are on the liver, kidneys, and central nervous system (CNS). Human symptoms of acute (short-term) inhalation and oral exposures to carbon tetrachloride include headache, weakness, nausea, and vomiting. Acute exposures to higher levels and chronic (long-term) inhalation or oral exposure to carbon tetrachloride produces liver and kidney damage in humans. Human data on the carcinogenic effects of carbon tetrachloride are limited. Studies in animals have shown that ingestion of carbon tetrachloride increases the risk of liver cancer. EPA has classified carbon tetrachloride as a Group B2, probable human carcinogen.

2.5.1 Uses of Carbon tetrachloride (CCl₄)

Carbon tetrachloride was produced in large quantities to make refrigerants and propellants for aerosol cans, as a solvent for oils, fats, lacquers, varnishes, rubber waxes, and resins, and as a grain fumigant and a dry cleaning agent. Consumer and fumigant uses have been discontinued and only industrial uses remain.

2.5.2 Sources and Potential Exposure to carbon tetrachloride

Individuals may be exposed to carbon tetrachloride in the air from accidental releases from production and uses, and from its disposal in landfills where it may evaporate into the air or leach into groundwater. (U.S. Environmental Protection Agency) Carbon tetrachloride is also a common contaminant of indoor air; the sources of exposure appear to be building materials or products, such as cleaning agents, used in the home. (U.S. Environmental Protection Agency) Workers directly involved in the manufacture or use of carbon tetrachloride are most likely to have significant exposures to carbon tetrachloride. (U.S. Environmental Protection

Agency) Individuals may also be exposed to carbon tetrachloride by drinking contaminated water. (U.S. Environmental Protection Agency)

2.5.3 Assessing Personal Exposure to carbon tetrachloride

Measurement of carbon tetrachloride in exhaled breath has been the most convenient method for determining exposure; measurements in blood, fat, or other tissues have also been used as indicators of exposure. However, these tests are not routinely available and cannot be used to predict whether any health effects will result. (U.S. Environmental Protection Agency)

2.5.4 Health Hazard Information

2.5.4.1 Acute Effects of CCl₄

Acute inhalation and oral exposures to high levels of carbon tetrachloride have been observed primarily to damage the liver (swollen, tender liver, changes in enzyme levels, and jaundice) and kidneys (nephritis, nephrosis, proteinuria) of humans. Depression of the central nervous system has also been reported. Symptoms of acute exposure in humans include headache, weakness, lethargy, nausea, and vomiting. (U.S. Environmental Protection Agency)

Delayed pulmonary oedema (fluid in lungs) has been observed in humans exposed to high levels of carbon tetrachloride by inhalation and ingestion, but this is believed to be due to injury to the kidney rather than direct action of carbon tetrachloride on the lung. (U.S. Environmental Protection Agency).

Acute animal exposure tests in rats, mice, rabbits, and guinea pigs have demonstrated carbon tetrachloride to have low toxicity from inhalation exposure, low-to-moderate toxicity from

ingestion, and moderate toxicity from dermal exposure. (U.S. Department of Health and Human Services).

2.5.4.2. Chronic Effects (Non-cancerous):

Chronic inhalation or oral exposure to carbon tetrachloride produces liver and kidney damage in humans and animals. (U.S. Department of Health and Human Services)

2.5.4.3 Reproductive/Developmental Effects of CCl₄

No information is available on the reproductive effects of carbon tetrachloride in humans. Limited epidemiological data have indicated a possible association between certain birth outcomes (e.g., birth weight, cleft palate) and drinking water exposure. However, as the water contained multiple chemicals, the role of carbon tetrachloride is unclear. (U.S. Department of Health and Human Services)

Decreased fertility and degenerative changes in the testes have been observed in animals exposed to carbon tetrachloride by inhalation. (U.S. Department of Health and Human Services). Birth defects have not been observed in animals exposed to carbon tetrachloride by inhalation or ingestion. (U.S. Department of Health and Human Services).

2.5.4.4 Cancer Risk of CCl₄:

Occasional reports have noted the occurrence of liver cancer in workers who had been exposed to carbon tetrachloride by inhalation exposure; however, the data are not sufficient to establish a cause-and-effect relationship. (International Agency for Research on Cancer).

CHAPTER THREE

3.0 Materials and Methods

3.1. Plant Material

Garcinia kola seeds were obtained from the Samaru Market in Zaria, Kaduna, Nigeria and was authenticated at the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria and a voucher number (1783) was obtained.

3.1.1 Extraction of plant material.

Preparation of the plant (*G.kola seeds*) extract was conducted in the department of Pharmacognocny, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

The method of maceration by Adaramoye, (2010) for the preparation of aqueous *G.kola* seed extract was employed.

3.2 Experimental Animals

Thirty-six (36) Wistar rats, male (24) and female (12), were purchased from the Pharmacology Animal House Centre, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria. They were housed in the Animal House of the Department of Human Anatomy and allowed to acclimatize for two weeks prior to the commencement of the experiments.

All the animals were given food (rat chow) and water ad libitum. Experimental groups were given aqueous extract in an amount of 400 mg/kg and 800 mg/kg, Duze *et al*, (2012.) in addition to a single dose of CCL₄. The experimental rats were weighed at the beginning, during and at the end of the study.

3.3 Experimental Design

A total number of 36 Wistar rats (male and female) were distributed randomly into **six** groups (six rats/group). The experiment lasted for a period of fourteen days during which **Group I** (positive control group) received distilled water, volume per body weight daily for 2 weeks; **Group II** (CCl₄ control group) received 0.4ml/kg on 'day 14'; **Group III** (standard control group) received 100mg/kg of silymarin for 2 weeks followed by a single dose of CCl₄ on day 14; **Group IV** (extract control group) received 800mg/kg (LD50 of AEGK is 6,741.43mg/kg, Duze *et al.*, 2011, Udenze *et al.*, 2012) for 2 weeks; **Group V** (prophylactic treatment group) received 800mg/kg daily for 2 weeks followed by a single dose of CCl₄ (0.4mls/kg) on day 14; **Group VI** (prophylactic treatment group) received 400mg/kg daily for 2 weeks followed by a single dose of CCl₄ (0.4ml/kg) on 'day 14'.

Table 3.1: Different experimental groups and their respective treatments.

Group	Treatment	Dose	Duration (days)	Route of administration
I	Distilled Water	-	14	Oral
II	<i>CCL₄</i>	0.4 ml/kg	Single dose (day14)	Intraperitoneal (IP)
III	<i>Silymarin</i> + <i>CCL₄</i>	100mg/kg + 0.4ml/ kg	14 + single dose (day 14)	Oral/IP respectively
IV	Extract	800 mg/kg	14	Oral
V	Extract + <i>CCL₄</i>	800mg/kg + 0.4ml/kg	14 + single dose (day14)	Oral/IP respectively
VI	Extract + <i>CCL₄</i>	400 mg/ kg + 0.4 ml/ kg	14 + single dose (day 14)	Oral/IP respectively

3.4 Sacrifice of Animals

At the end of the experiment (day 15), all the animals were humanely sacrificed. Blood was collected through the jugular vein of the animals in heparinized centrifuge tube under a deep anaesthesia with chloroform. The blood collected was centrifuged using centrifuge machine at 10,000 rpm for ten minutes and the serum collected was subjected to liver function test (AST, ALT and ALP) and estimation of oxidative stress enzymes (SOD, CAT and GSH). The liver tissue was harvested for histological examination.

3.5 Morphological Studies

The experimental animals were weighed daily, before, during and after the experiment. After the experimental period, the animals were humanely sacrificed following chloroform anaesthesia. The liver tissues were harvested and fixed in 10 % formalin for histopathological analysis.

3.6 Biochemical Studies

3.6.1 Liver function Test

The liver enzymes analysis; alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), alkaline phosphatase (**ALP**) was carried out using an auto-analyzer and the ALT: AST ratio was determined.

3.6.2 Estimation of Oxidative Stress Enzymes

3.6.2.1 Superoxide Dismutase

Superoxide dismutase (**SOD**) was carried out according to the method described by Fridovich (1989).

3.6.2.2 Procedure

The ability of superoxide dismutase (SOD) to inhibit auto oxidation of adrenaline at pH 10.2, form the bases of this assay. 0.05 M phosphate buffer: 6.97 g of diphosphate K₂HPO₄ and 1.36 g of KH₂PO₄ was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 7.8. 0.05 Carbonate buffer: 14.3g of Na₂CO₃) and 4.2g Of NaHC0₃) was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2. 0.3 mM Adrenaline: 0.01 g of adrenaline was dissolved in 17 ml of distilled water. The solution was prepared fresh. 0.1 ml of microsome will be diluted in 0.9 ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.20 ml of the diluted microsome was added to 2.5 ml of 0.05 M Carbonate buffer. The reaction started with the addition of 0.3ml of 0.3 mM adrenaline. The reference mixture contains 2.5 ml of 0.05 M Carbonate buffer. 0.3ml of 0.3mM adrenaline and 0.20 ml of distilled water. Absorbance was measured ever 30 up to 150 s at 480nm. 1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

5.0 ml of R1 was put in test tube 1 i.e. (sample blank) while to the sample test tube 2 (serum) was added 0.5 ml R1 and 0.1 ml of serum. This was incubated at 37° for 30mins. After this, 0.5 ml of R2 was then added to all test tubes, in addition to 0.1 serum to the sample blank test tube only. This was allowed to stand for 20mins at 25° C. Finally, 5.0 ml of sodium hydroxide was

added to both test tubes, content mixed and the absorbance of the sample (A_{sample}) was read (at 546 nm wavelength) against the sample blank after 5 mins. This was done for all samples obtained from the experimental animals.

3.6.2.3 Catalase (CAT)

Assay for catalase activity was assayed according to the method of Machly and Chance (1954).

3.6.2.4 Principle

Catalase can act on H₂O₂ to yield H₂O and O₂. The concentration of H₂O₂ in the initial and final absorbance reading after 10 minutes was determined as catalase activity.

3.6.2.5 Reagents

1. 0.01M phosphate buffer (pH 7.0) was prepared by weighting 0.142 g of distilled water.
2. 0M H₂O₂ was prepared by diluting 11.33ml of 6 % w/v of H₂O₂ in 10 ml of distilled water.
3. 5 % potassium dichromate prepared by dissolving 5 g of potassium dichromate in 100 ml distilled water.
4. Potassium dichromate and glacial acetic acid solution on 1:3 ratio was prepared by diluting 5 ml of 5 % potassium dichromate with 15 ml of glacial acetic acid.

3.6.2.6 Procedure

To 10 µl (serum sample), 50 µl 0.1M of phosphate buffer (pH = 7.4) was added with 20 µl of H₂O₂. The reaction was then stopped by the addition of 100 µl of potassium dichromate and glacial acetic and reagent. The initial and final absorbance was measured at 620 nm at 10 minutes interval.

3.6.2.7 Calculation

The calculation was done using the Beer's Law to determine the activity of the catalase enzyme as shown below:

$$A = \epsilon CL$$

where A = Absorbance

ϵ = Molar extinction coefficient

C = Concentration

l = Path length of the cuvette

For t_0 $C_1 = A/\epsilon l$

or $C_2 = A/\epsilon l$

The amount of the substrate converted after one minute was found to be.

$$C_2 - C_1.$$

$$C = \text{mm/ml}$$

1. $\frac{\text{Absorbance of the blank} \times 1000}{\text{Absorbance of sample}}$

3.6.3 Reduced glutathione (GSH)

Assay of reduced glutathione (GSH) concentration measurement was done according to Ellman (1959) as described by Rajagopalan *et al.* (2004).

3.6.3.1 Reagents

0.2M phosphate buffer: 8.40g of NaH_2PO_4 and 9.94 of Na_2HPO_4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 8.

3.6.3.2 Procedure

To 150µl of serum or tissue homogenate (in phosphate-saline PH 7.4), 1.5ml of 10% TCA was added and centrifuged at 1500g for 5 mins. 1 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5' – dithiobis (nitro benzoic acid) (DNTB) in 100ml of 0.1 % sodium nitrate) and 3 ml of phosphate buffer (0.2 M, pH 8). The absorbance was read at 412 nm.

3.6.3.3 Measurement of glutathione reductase (GSH Rd) activity.

GSH Rd activity was measured according to the procedure of Ellman (1959).

Procedure

Briefly, 10µL of cell homogenate was added to a reaction mixture (containing 0.99 mL of 100 mM potassium phosphate buffer pH 7.0, 1.1 mM MgCL₂, 5 Mm GSSG and 0.1 mM NADPH) to trigger the Nicotinamide adenine Dinucleotide Phosphate reduced (NADPH) conversion reaction. Changes in absorbance were monitored by a continuous-recording spectrophotometer at 340nm for 5 min at 25° C.

3.7 Histopathological Analysis

Histological study was performed following a midline laparotomy to remove the liver. The liver tissues were immediately fixed in 10% buffered formalin and subsequently embedded in paraffin. Liver sections were processed for routine histological staining applying the Haematoxylin and Eosin (H and E) technique for the general hepatic architecture, Gordon and Sweet (G and S) stain for reticular fibres using standard techniques and sections were viewed

under the light microscope. Photomicrographs were obtained using a digital microscope (Leitz-Witzlar) with its monitor.

3.8 Data Analysis

Results obtained were analysed using the statistical software, Statistical Package for Social Scientist (SPSS *version 18.0*) and results were expressed as mean \pm SEM. Differences among means of the groups were determined using one way ANOVA with *LSD post hoc test*. Paired sample *t*-test was also used as appropriate and values were considered statistically significant when $p < 0.05$.

CHAPTER FOUR

4.0 Results

4.1 Physical Observation of Animals.

During the period of administration of the aqueous extract of *Garcinia kola* seed, all the experimental animals (Wistar rats) were observed to exhibit normal physical activity. Weight changes were observed in the experimental animals *before* (day 1) and *after* (day 14) the experiment, with their weight differences.

Table 4.1 shows body weight changes in experimental animals using one way ANOVA. Weight changes were observed in the groups. Increase in body weights of animals were observed in group I (control), group III (Sil + CCl₄), group IV (extract high) and group VI (extract low + CCl₄). However, results revealed that animals in groups II (CCl₄) and V (extract high + CCl₄) exhibited decrease in body weight.

There was significant decrease ($p < 0.05$) in the body weight of group II animals when compared to control. However, results revealed significant increase ($p < 0.05$) in body weights of animals in groups III, IV, and VI when compared to CCl₄.

Table 4.1 Effect of *Garcinia kola* aqueous extract, silymarin, and CCl₄ administration on the body weight of Adult Wister rats.

Treatment/ Group	Day 1(before)	Day 14(after)	Weight Differences(g)
Control (I)	188.50±0.04	203.25±0.01	13.6±3.57
CCl₄ (II)	302.60±0.10	294.60±0.05	-15.60±4.17**
Sil+CCl₄ (III)	214.80±0.01	218.80±0.04	4.00±2.28*
EX High (IV)	186.40±0.04	198.00±0.01	15.60±3.68**
EX High + CCl₄ (V)	262.60±0.07	256.80±0.14	-3.60±9.01
EX Low + CCl₄ (VI)	223.60±0.01	231.40±0.01	7.80±4.02*

n=6, mean ± SEM; One way ANOVA, *= p<0.05, **= p<0.01. CCl₄=carbon tetrachloride, SIL=Silymarin (100mg/kg), EX High = *Garcinia kola* aqueous extract (800mg/kg), EX low = *Garcinia kola* aqueous extract (400mg/kg).

4.2 Biochemical Analysis

4.2.1 Liver Enzymes: AST, ALT and ALP

Table 4.2 revealed that the CCl₄ treated group (group II) expressed remarkably high levels of liver enzymes (aspartate aminotransferase, AST; Alanine Phosphatase, ALP and Alanine aminotransferase, ALT). There was decrease in ALP level at extract high dose treatment when compared to control; however, this was not significant. Rats that received a single dose of CCl₄ (group II) showed marked elevation in the levels of liver enzymes when compared with that of the control (group I) rats. Pretreated groups III, IV, V and VI rats revealed significant increase in the levels of AST and ALT. However, there was decrease in ALP level significant with silymarin + extract low dose administration when compared with group II rats that received CCl₄ alone.

There was a significant increase in the AST: ALT ratio obtained in the group II animals when compared to the control (group I).

Table 4.2 Biochemical Assay of Liver Enzymes following oral administration of *Garcinia kola* seed aqueous extract in CCl₄ induced rats.

Group	AST (u/l)	ALT (u/l)	ALP (u/l)	AST:ALT
Control	45.00±09.72	56.25±04.17	434.25±22.83	0.80
CCl₄	179.60±09.94***	123.86±10.29***	525.00±30.25	1.45***
Sil + CCl₄	136.75±11.83***	95.87±07.98**	289.50±21.14*	1.43***
EX High	88.25±11.27**	89.14±06.49**	357.75±40.48	0.99*
EX High + CCl₄	148.25±06.14***	120.25±36.22**	509.50±84.25	1.23***
EX Low + CCl₄	133.75±03.71***	95.25±15.36**	212.75±45.35**	1.40***

n=6, mean±SEM; Sil= silymarin, EX= *G. kola* seed aqueous extract, AST= Aspartate aminotransferase, ALT= Alkaline aminotransferase, ALP= Alkaline phosphatase. *= p< 0.05, **=p< 0.01, ***= p<0.000 when compared to control.

4.2.2 Oxidative stress indicator

Results depicted in table 4.3 showed that administration of *Garcinia kola seed* aqueous extract caused insignificant increase in the levels of SOD and GSH as compared to the low levels observed in the CCl₄ treated group. However, there is also no significant difference in the increase of the catalase levels when compared to CCl₄ group.

In the liver tissue, increased levels of lipid peroxides were recorded in the group II rats. The activities of the peroxides decreased with increase in the amount of the *Garcinia kola seeds extract* administered. This is evidenced in the values obtained in rats in groups V, and VI.

Table 4.3 Serum levels of catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) on the effect of *garcinia kola* seed aqueous extract on oxidative stress markers in CCl₄ induced hepatotoxicity.

	CAT(U/mg)	GSH (nmol/mg)	SOD (U/mg)
Control	0.302±0.12	0.228±0.05	0.087±0.01
CCl₄	0.137±0.05	0.130±0.05	0.067±0.02
Sily. + CCl₄	0.452±0.19	0.234±0.05	0.088±0.01
EX High	0.338±0.07	0.238±0.04	0.093±0.01
EX High + CCl₄	0.252±0.59	0.207±0.05	0.090±0.02
EX Low + CCl₄	0.353±0.02	0.221±0.03	0.090±0.01

n=6, mean ± SEM; Sil= silymarin, EX= *G. kola* aqueous seed extract, catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD).

4.3 Histological Studies of Liver

4.3.1 Histological Features of the Control (Untreated) Experimental Animals

Liver sections of control group showed normal histoarchitecture of the liver parenchyma; the characteristic appearance of the hepatic cells (hepatocytes) having preserved cytoplasm, prominent nuclei and nucleoli, and hepatocytes radiating from the central vein. Fine vascular spaces separate the thin plate of hepatocytes, the sinusoids (Plate 1).

4.3.2 Histological Features of the Treated Experimental Animals

Liver sections of the CCl₄ treated group (group II) showed distortion in the histoarchitecture of the liver parenchyma, such as, areas of vacuolar degeneration, necrotic cells, and congestion (blood cells in the hepatocytes). Infiltration of inflammatory cells, around the blood vessels, was also observed (Plate 2). Liver sections of Wistar rats of the standard drug (silymarin) group (group III) showed normal liver histoarchitecture with few inflammatory cells seen (Plate 3).

Sections of liver of the extract (*G. kola seed*) in the treated group (extract control; group IV) showed the histology of liver parenchyma comparable to that of the control group with normal hepatocytes with preserved cytoplasm, prominent nuclei, and nucleoli and central vein (Plate 4).

Administration of *Garcinia kola seed* extract preserved the histoarchitecture of the liver against CCl₄ administration, at low and high doses. Mild vacuolar degeneration was observed in the liver sections of Wistar rats treated with high dose extract (Plate 5). Comparatively, the low extract dose presented a better hepatoprotective effect on the histology of the liver (Plate 6).

4.3.3 Reticular Fibres

Liver sections from the control group (group I) showed normal histoarchitecture of the liver parenchyma with distinct intensely stained (G and S stain) reticular fibres well drawn out from the central vein (Plate 7). Liver sections of the CCl₄ - treated group (group II) showed distortion of the histoarchitecture of liver as greatly distorted reticular fibres arrangement (Plate 8). Liver section of the standard drug treated group (group III), revealed preserved reticular fibres, with mild distortion of the reticular fibres, but not as severe as when compared to the CCl₄ treated group (Plate 9).

Liver sections of the extract control group (treated with high dose extract only; group IV), the reticular fibres were found in place, radiating spirally out from the central vein into the liver tissue and these were comparable to those of the control group (Plate 10).

Plates 11 and 12, liver sections of the high dose and low dose extract treated groups respectively, revealed preserved reticular fibres, with mild depletion compared to the CCl₄ control group.

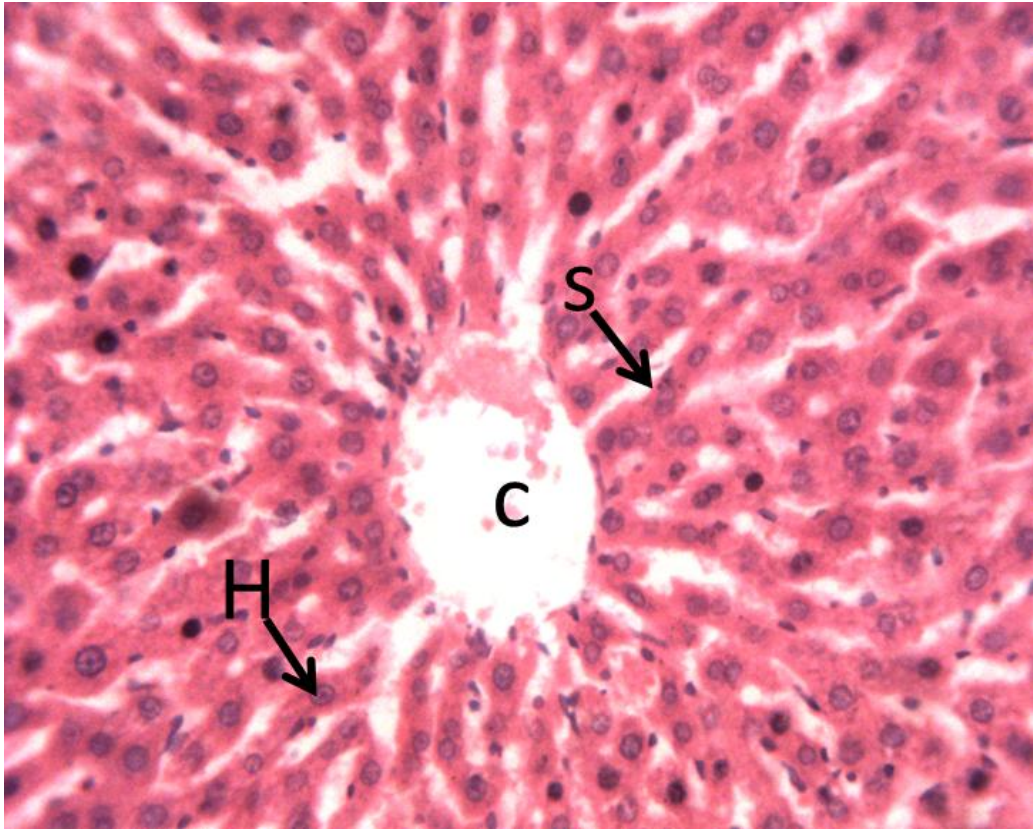


Plate 1: Liver section of control group I showing normal liver histoarchitecture; Central vein (C); Hepatocyte (H); Sinusiod (S) (H and E, x 250).

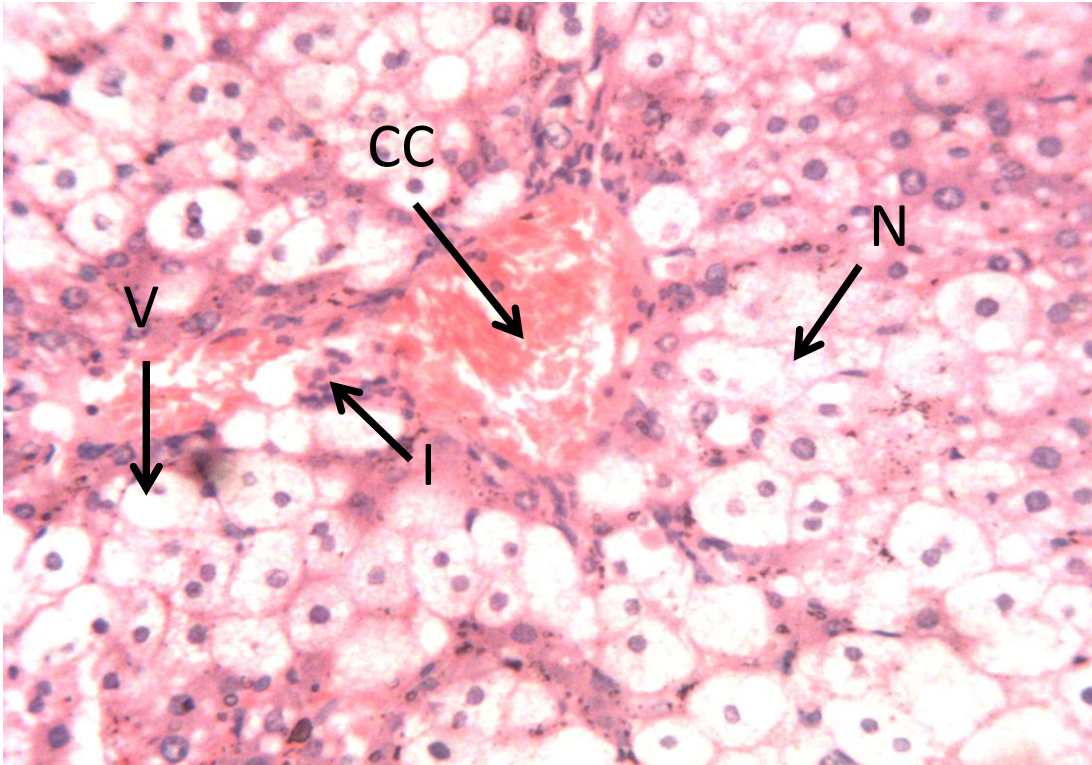


Plate 2: Liver section of CCl_4 treated group II showing distorted liver histoarchitecture; Congested Central vein (CC); Infiltration of inflammatory cells (I); Necrosis (N); Vacuolar degeneration (V), (H and E x 250).

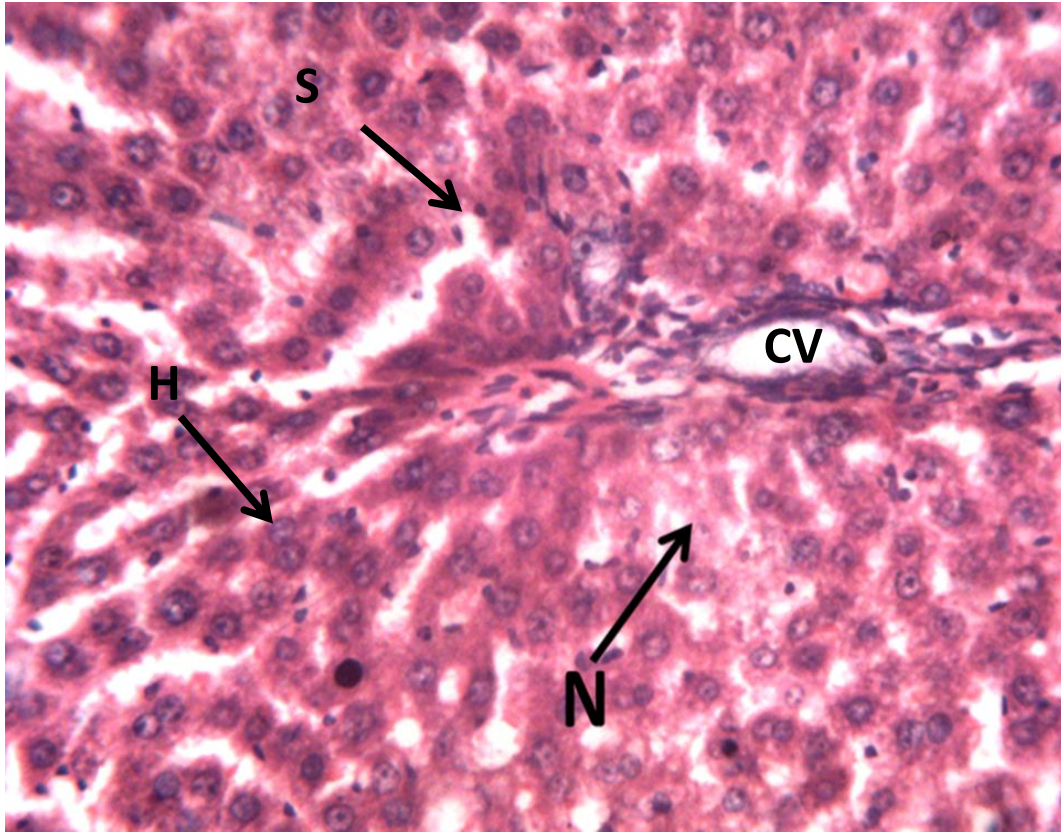


Plate 3: Liver section of Silymarin and CCl₄ treated group III showing mild distortion of the liver histoarchitecture; Hepatocytes (H), Central vein (CV), sinusoids(S) with few necrotic cells (N); (H and E x 250).

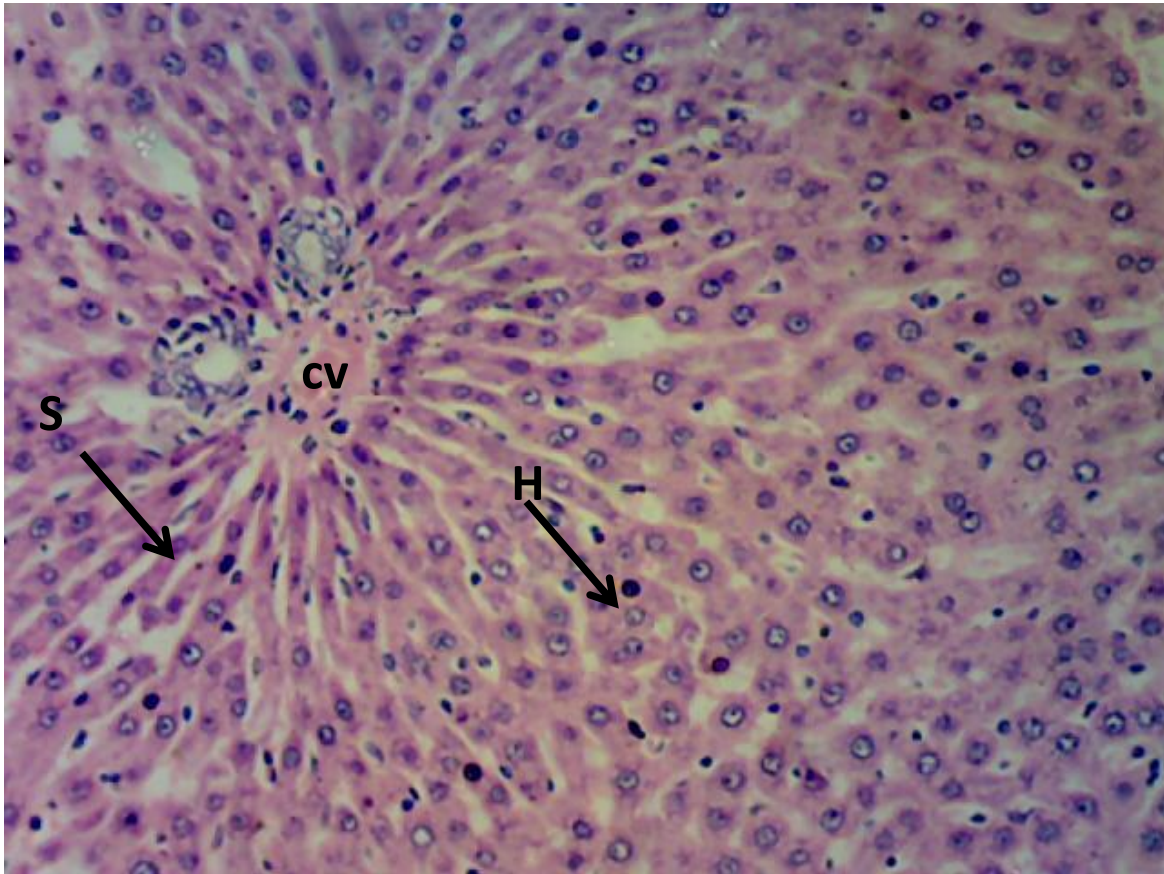


Plate 4: Liver section of extract (800mg/ kg) treated group IV showing the liver histoarchitecture; Hepatocyte (H); Sinusoid (S) (H and E x 250)

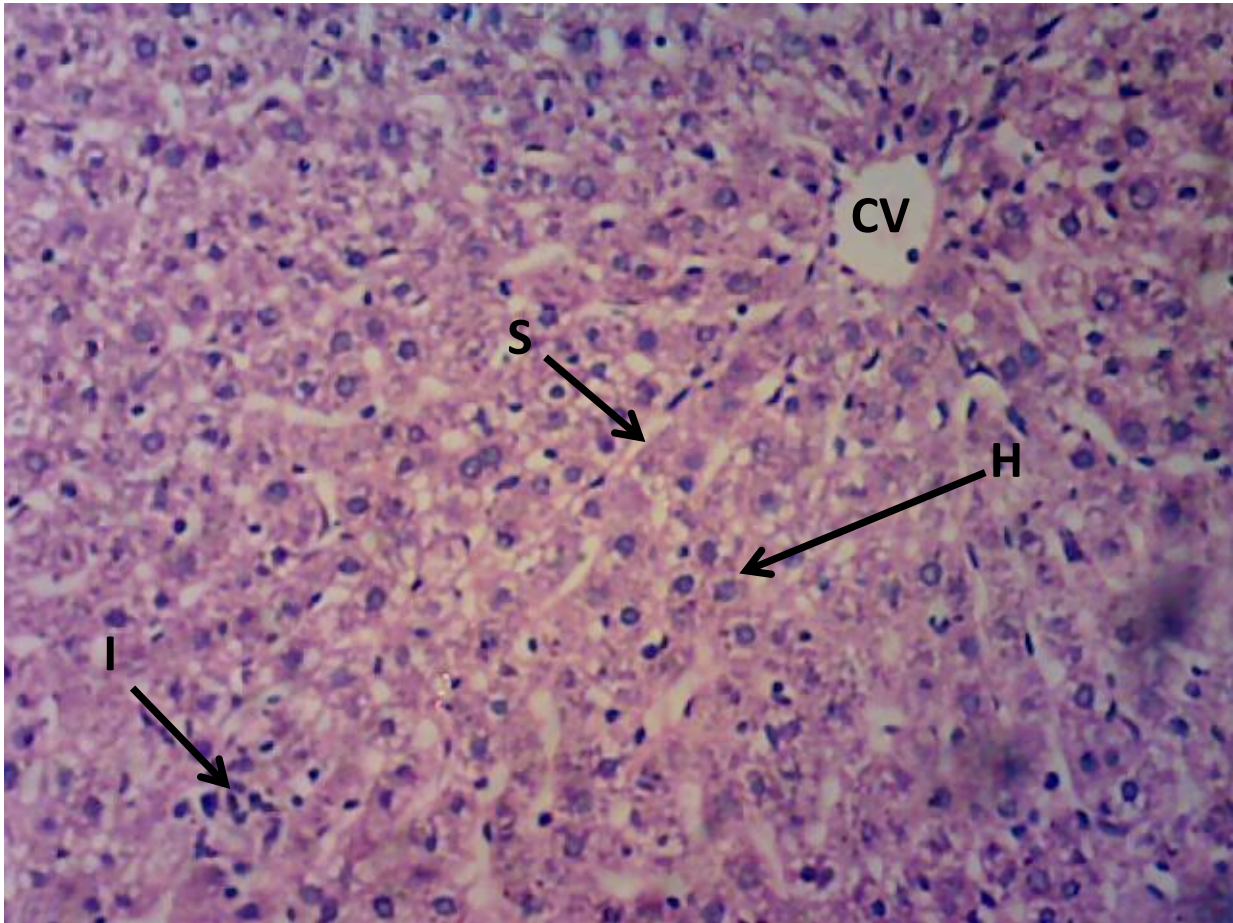


Plate 5: liver section of extract (800mg/kg) and CCl₄ treated group showing mild distortion of the liver histoarchitecture; hepatocyte (H); Sinusoid (S), Infiltration of inflammatory cells (I) (H and E x 250).

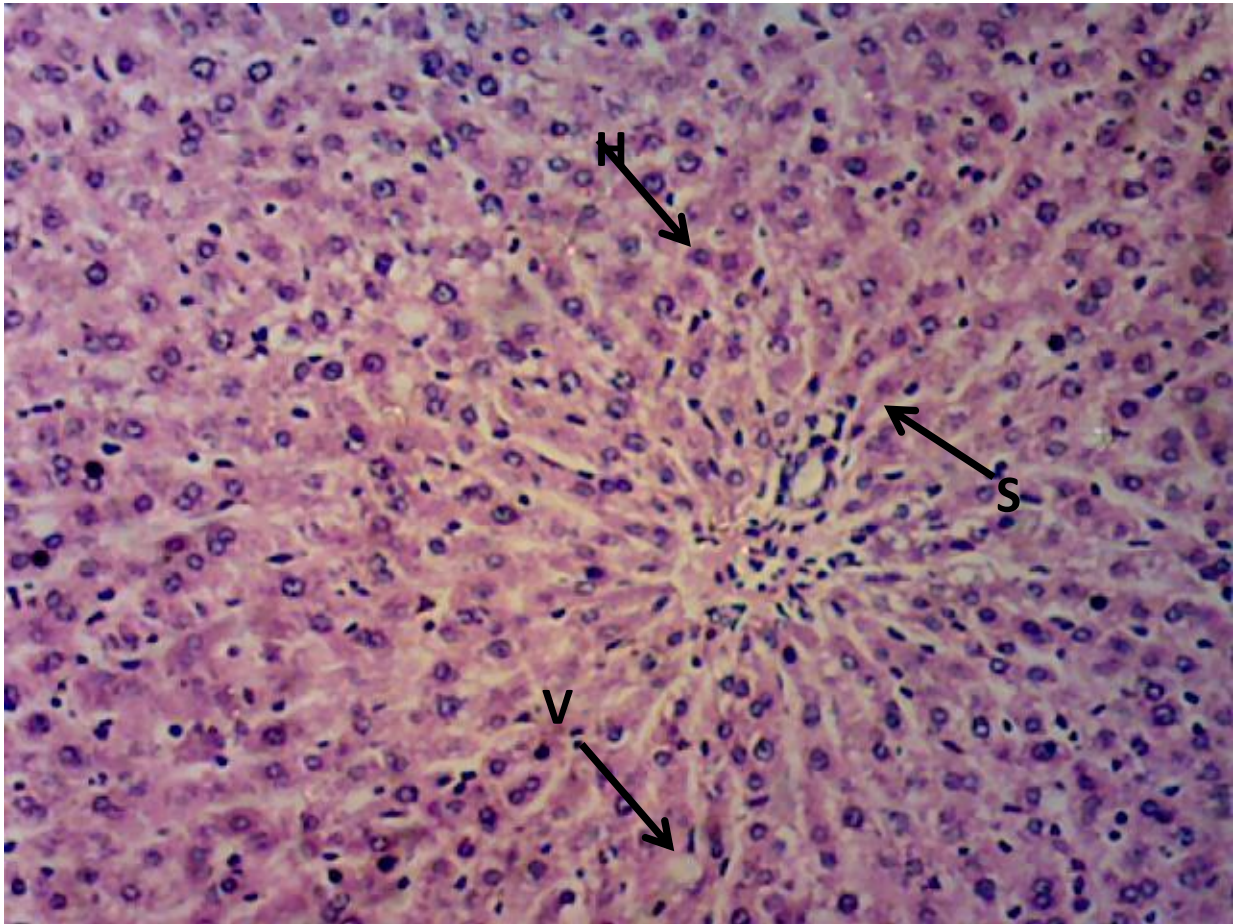


Plate 6: liver section of extract (400mg/kg) and CCl₄ treated group showing preservation of the liver histoarchitecture; hepatocyte (H), Sinusoid (S), (H and E x 250).

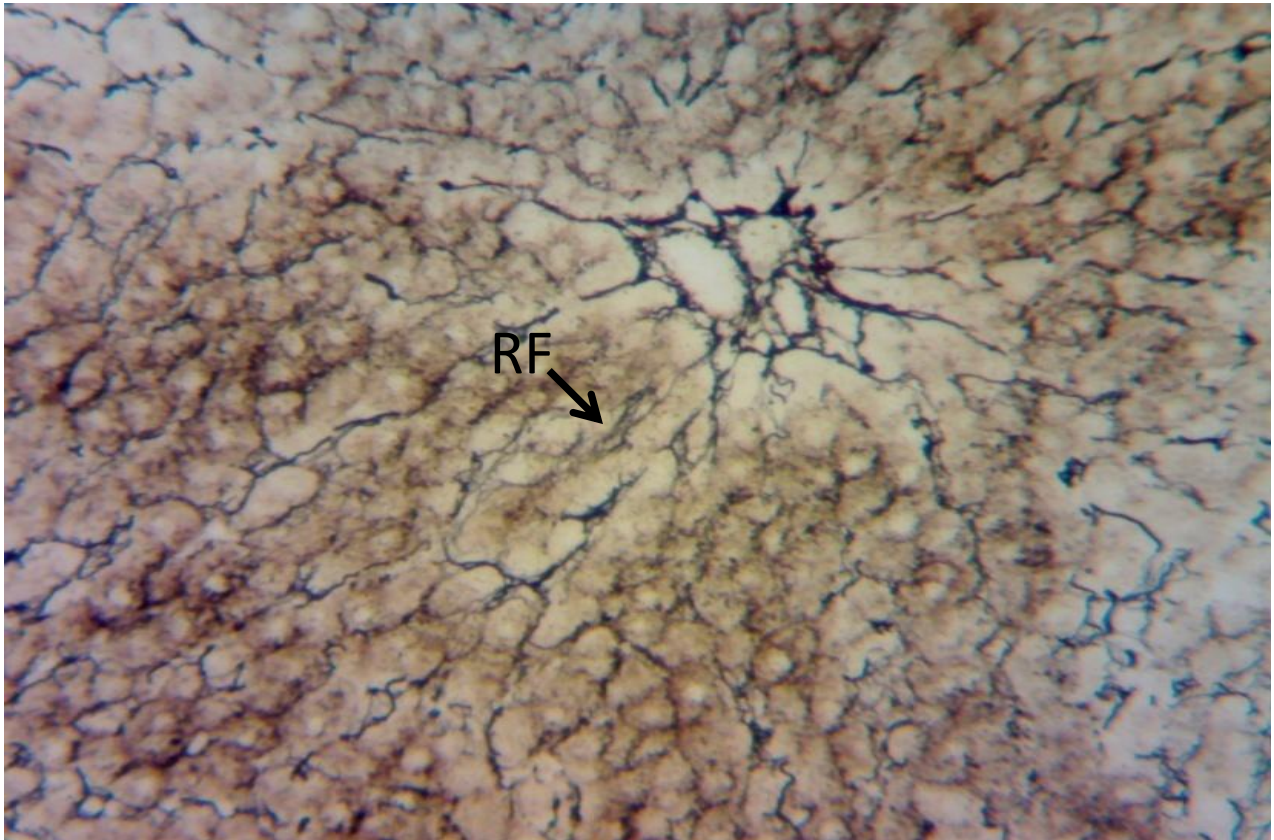


Plate 7: Liver section of control group I showing normal liver histoarchitecture; Reticular Fibre (RF) (G and S x 250).

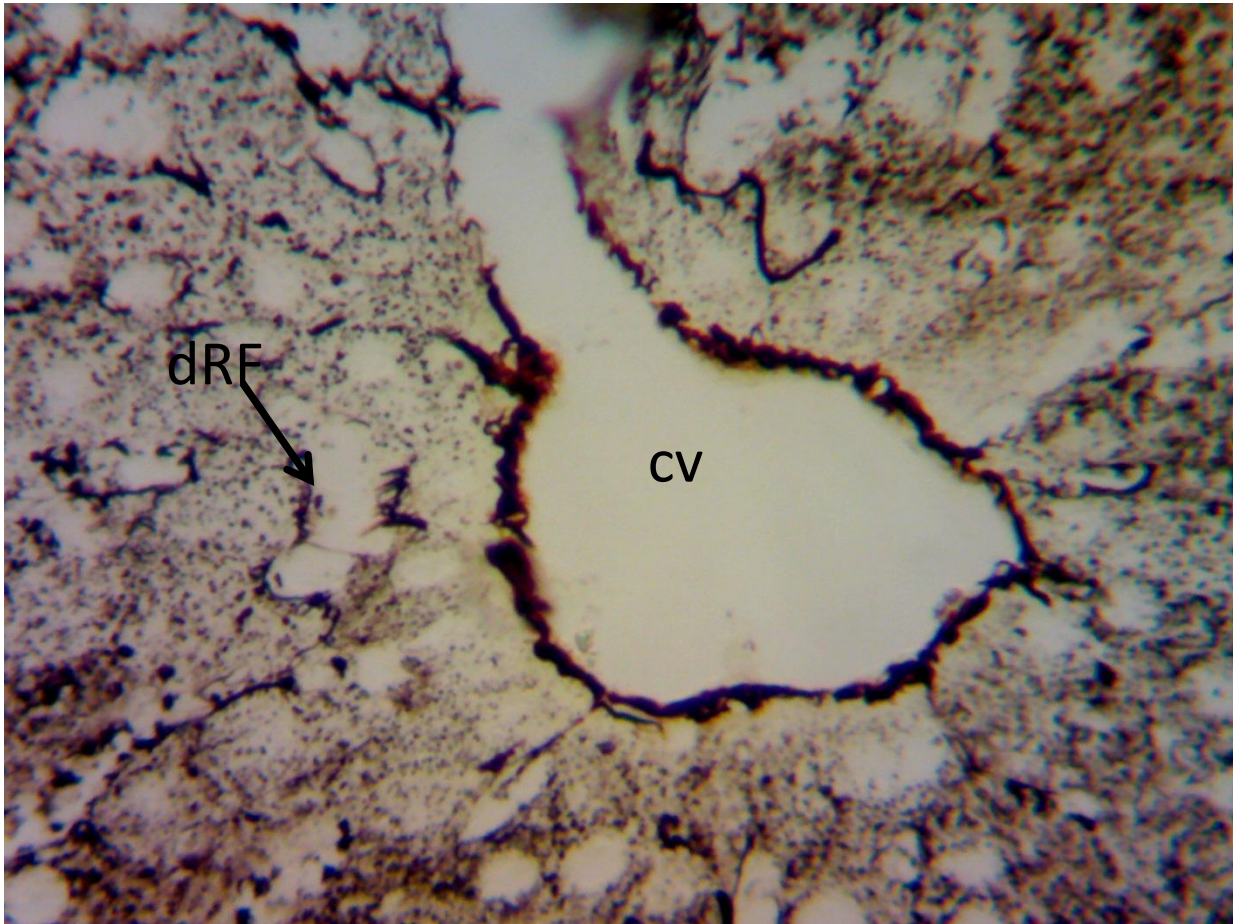


Plate 8: liver section of CCl₄ treated group showing distorted liver histoarchitecture; depleted Reticular fibre (dRF) (G and S x 400).

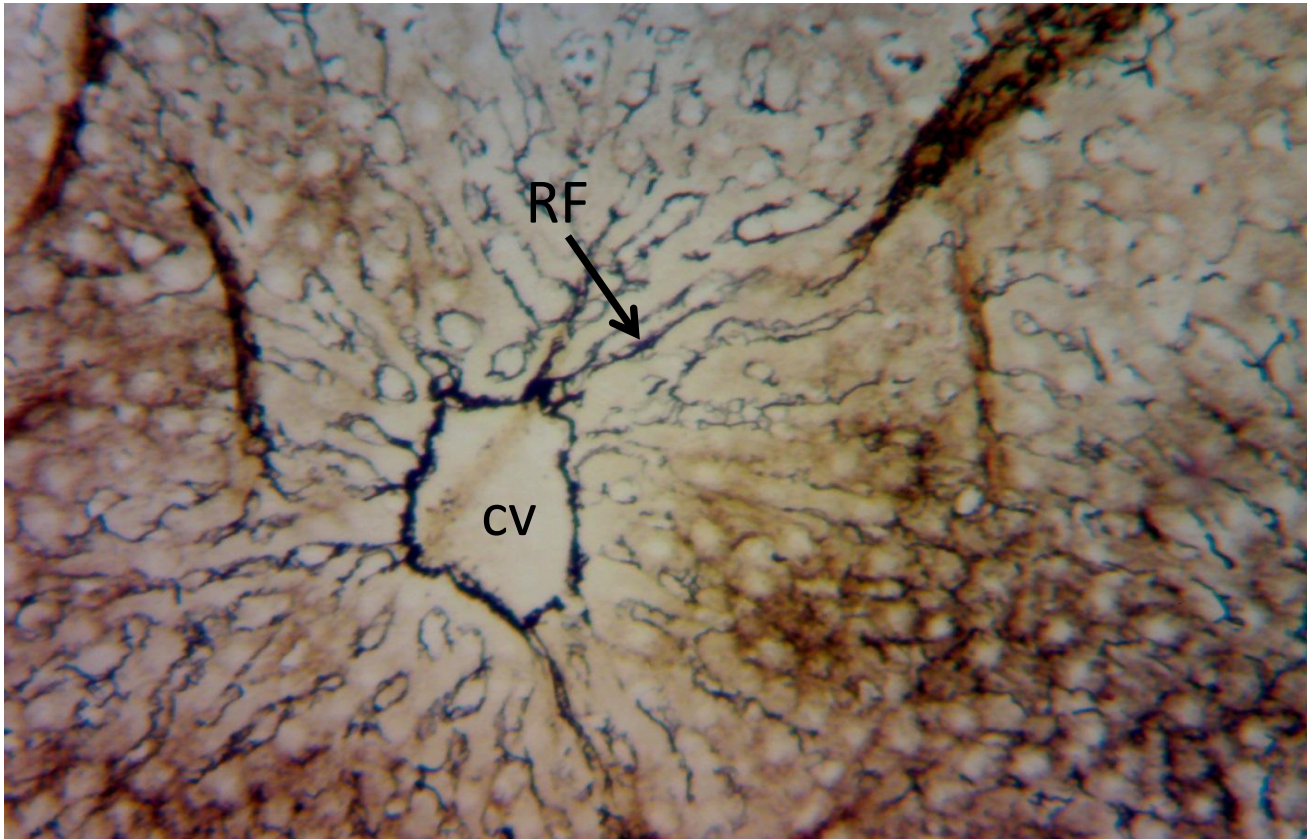


Plate 9: Liver section of Silymarin and CCl_4 treated group III showing mild distortion of the liver histoarchitecture; Central vein (CV), preserved reticular fibre (RF), (G and S x 250).

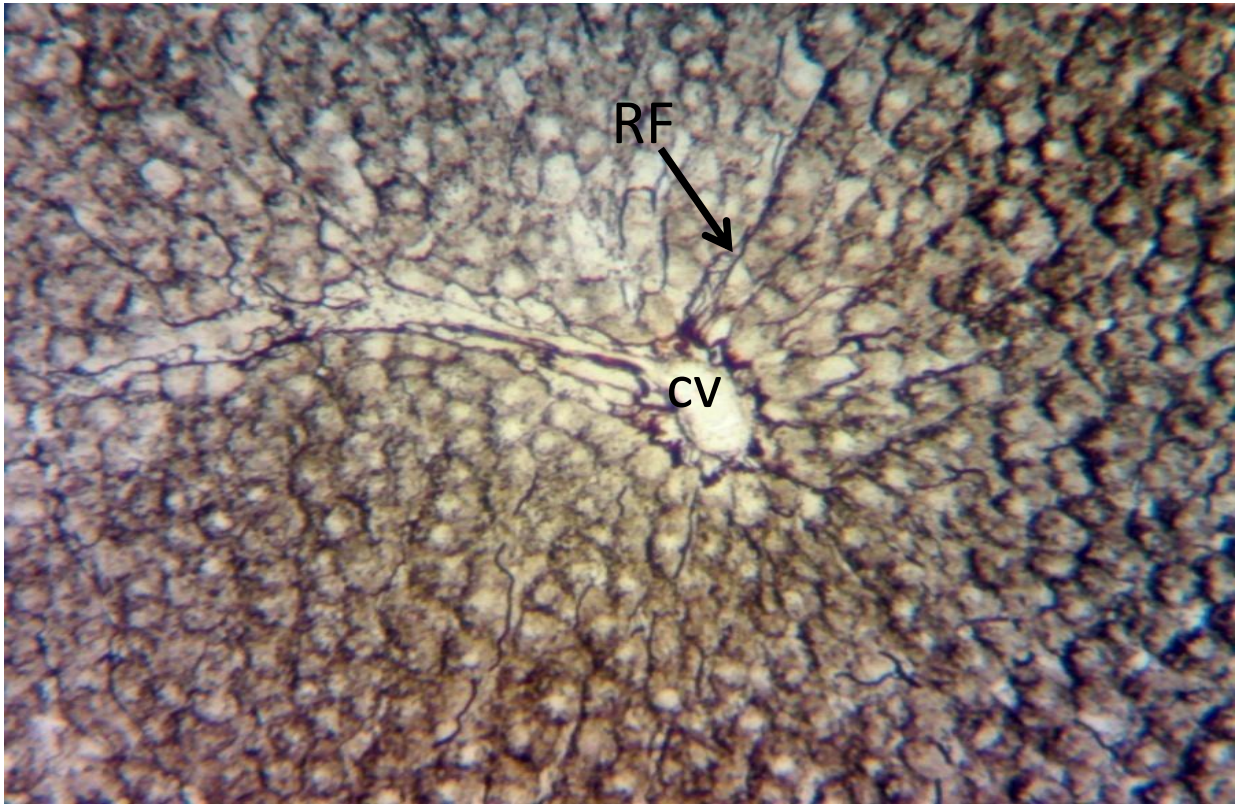


Plate 10: liver section of extract (800mg/kg) treated group showing the liver histoarchitecture; Central vein(C), preserved Reticular fibre (RF) (G and S x 250).

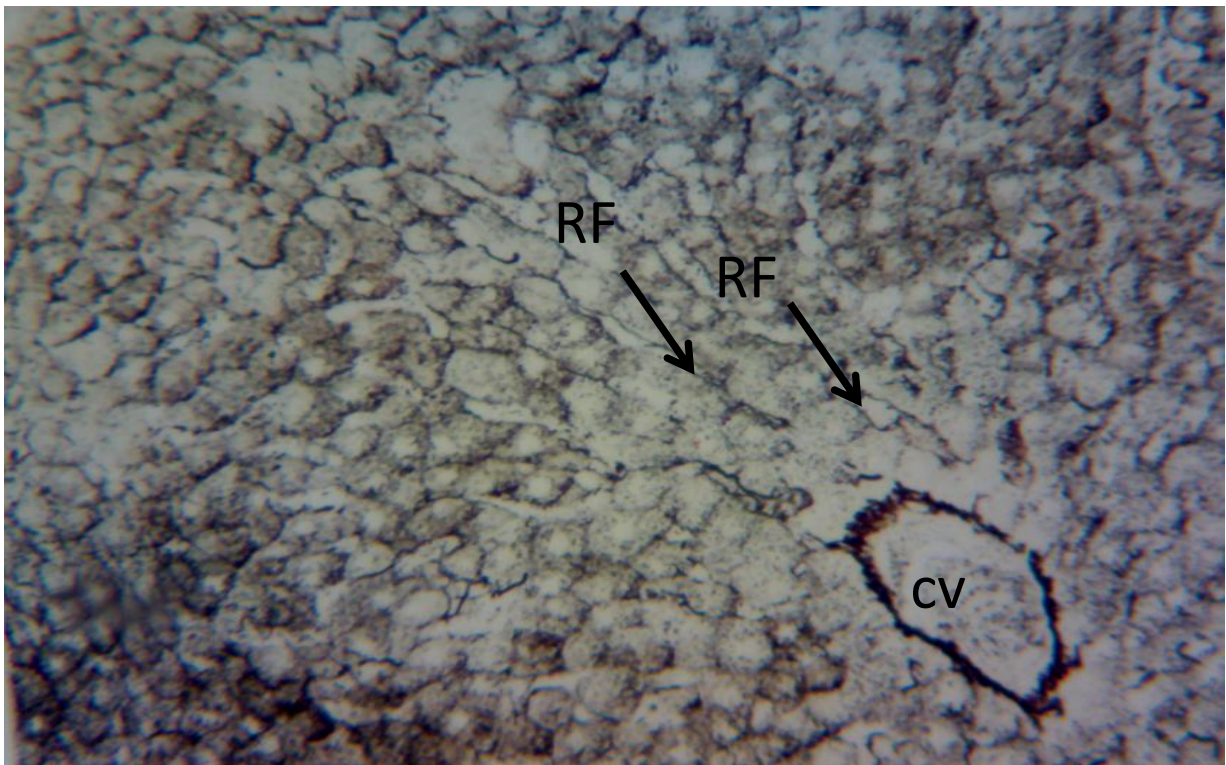


Plate 11: liver section of extract (800mg/kg) and CCl₄ treated group showing mild distortion of the liver histoarchitecture; Reticular fibre (dRF) (G and S x 250).

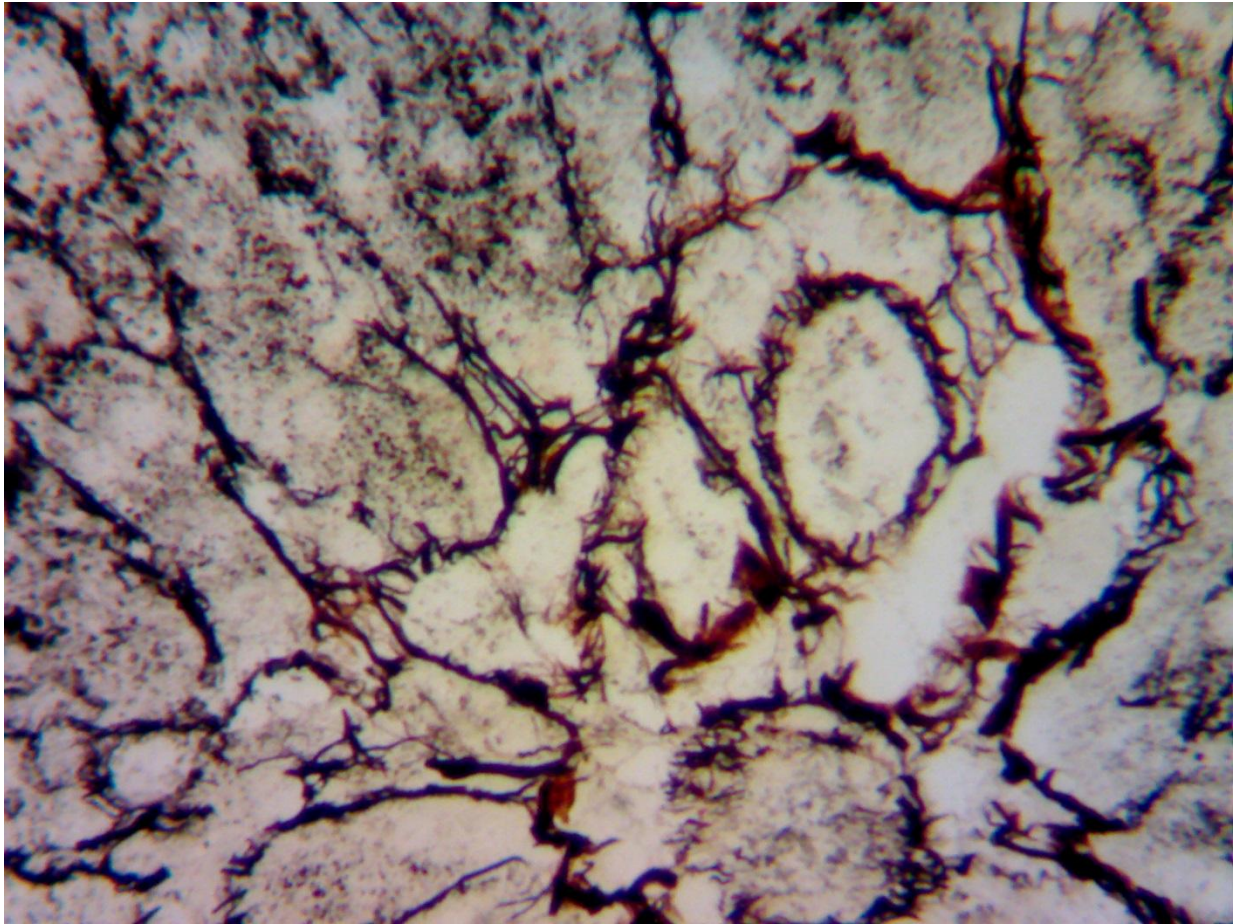


Plate 12: Liver section of extract (400mg/kg) and CCl₄ treated group showing mild distortion of the liver histoarchitecture; Central vein(C); R. fibre (dR) (G and S x 400).

CHAPTER 5

5.0 Discussion

5.1 Physical Observation

Changes in body weight have been used as an indicator of adverse effect of drugs and chemicals (Mukinda and Syce, 2007). Physical observation of the experimental animals (Wistar rats) revealed changes in the body weights. Marked decrease in the body weight observed in the CCl₄ treated group (group II) was as a result of the toxic effects of CCl₄ administration. This is in agreement with previous studies on the toxic effect of CCl₄ (Obi *et al.*; 1998; Nevien, 2012).

The observed increase in weight of group I (control) animals is weight increase due to growth. Decrease in body weights of animals treated with CCl₄ (group II) and high dose extract + CCl₄ (group V) suggests treatment related intoxication. This can also be attributed to the fact that animals in these groups might have reached their peak age (adults), hence the decline in weight.

Observed increase in body weights of extract low + CCl₄ treated group (group VI) suggest that the extract is partially potent against CCl₄ toxicity. Several studies in relation to herbal and folk medicine reported the hepatoprotective effect of plants against CCl₄ – induced hepatotoxicity (Prakesh *et al.*, 2008; Biswas *et al.*, 2010; Osman *et al.*, 2011; Sahreen *et al.*, 2011). This finding is in accordance with the report on the hepatoprotective potential of *Garcinia kola* extract against CCl₄ toxicity (Adaramoye, 2010).

The marked increase in body weight of the extract high group (group IV) shows the extract is safe at this dose (800mg/kg) and does not affect body weight. Reports on the acute toxicity of *G. kola* stated non-toxicity at a dose greater than 900mg/ kg and a lethal dose of 6741.43mg/ kg

(Duze *et al.*, 2011; Udenze *et al.*, 2012). Observed increase in body weights of the group treated with silymarin + CCl₄ implies the protective effect of silymarin as a standard hepatoprotective drug (Prakesh *et al.*, 2008).

5.2 Biochemical Analysis

5.2.1 Liver Enzymes (ALT, AST and ALP)

As elicited by the elevation in the levels of liver marker enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), CCl₄ administration resulted in a significant hepatic damage. Obviously, the elevated levels of these biochemical parameters are indicators and a direct reflection of alterations hepatic structural and functional integrity (Obi *et al.*, 1998; Sahreen *et al.*, 2011). Elevation of liver enzymes levels obtained in this study corroborates with reports of previous studies in relation to CCl₄ toxicity; toxicity in liver due to CCl₄ and other chemicals is attributed to the toxic metabolites formed (Arulkumaran, 2007; Gnanaprakash *et al.*, 2010). ALT is more specific to the liver and thus a better parameter for detecting liver injury (Williamson *et al.*, 1990; Ojo *et al.*, 2006). Remarkable ($p < 0.01$) elevation of ALT enzyme level observed with CCl₄ administration in group II animals is indicative of liver damage. These enzymes are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged (Iroanya *et al.*, 2012).

There is a growing consensus among workers that CCl₄- induced liver damage occurs by the production of a trichloromethyl radical from CCl₄ when it is reductively dechlorinated. The trichloromethyl radical production attracts a hydrogen atom from fatty acid to form a lipid radical that reacts with molecular oxygen. The product of such reaction is the initiation of lipid peroxidation. There was significant decrease in ALP level with administration of silymarin.

Usmani and Kushwaha (2010) reported decreased levels of ALP with the administration of silymarin against elevated levels induced by CCl_4 administration. This implies that silymarin had a hepatoprotective effect on the liver enzymes, which is in agreement to the reports on the effect of the plant in the liver (Ferenci *et al.*, 1989; Usmani and Kushwaha, 2010; Osman *et al.*, 2011).

Since the above mechanism is suggestive of the process of oxidative stress, it is true, therefore, that any natural product with antioxidant property will prevent or reverse lipid peroxidation; including cell membrane damage (Ulicna *et al.*, 2003; Osman *et al.*, 2011). The report of Iwu, (1982) that implicated seeds of *Garcinia kola* in folk medicine and herbal preparations for treatment of liver disorders, informed the screening of its natural antioxidant properties. It could also be said that a comparison of the results obtained with that of silymarin (rated as one of the most powerful antioxidants) would positively influence the position on its antioxidant status. The findings in this study show that pretreatment of rats 14 days prior to CCl_4 administration caused a marked decrease in the levels of hepatospecific serum enzymes. This suggests that seeds of *Garcinia kola* may be protective against CCl_4 - induced liver damage in rats. This was ascertained by a comparative analysis of the results obtained in rats pretreated with *Garcinia kola*.

Furthermore, the AST/ALT ratio has been reported to provide information on the extent and differentiate between causes of liver damage (hepatotoxicity) Nyblom *et al.*, 2004. It has been reported that the normal ratio is less than 1, and when it is more than 1 but less than 2, it is said to be due to toxins, cirrhosis. When the ratio is greater than 2, it is more likely to be associated with alcoholic hepatitis (Sorbi *et al.*, 1999) or hepatocellular carcinoma.

The AST:ALT ratios obtained in the results (table 4.2) showed that were higher in the CCl₄ group (II) as compared to the control group (I) and the other treatment groups. This confirms the presence of toxicity due to chemical (CCl₄) in the group. The AST:ALT ratios obtained in the extract groups (IV, V and VI) and silymarin group (III) revealed values lower than the CCl₄ group suggesting that aqueous extract of bitter kola (*Garcinia kola*) and silymarin had a protecting effect on the liver enzymes by lowering the AST:ALT ratios to near normal.

5.2.2 Oxidative Stress Indicators

Antioxidant enzymes and lipid peroxidation levels can be used to predict the severity of drug induced liver damage (Ogunlade *et al.*, 2012). Antioxidants enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) and Reduced glutathione (GSH) dependently act in the metabolic pathways that involve free radicals. Therefore changes in antioxidant enzymes activity, oxidative stress markers (CAT, SOD, and GSH) are indicators of liver damage (Sahreen *et al.*, 2011; Nevein, 2012). Extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of poly-unsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and the death of cell. The decrease in CAT, SOD, and GSH is due to the toxic effect of CCl₄.

Decreased antioxidant enzymes activity (CAT, SOD, and GSH) observed in CCl₄ treated group (group II) reflect a treatment related toxicity. This is in agreement with reports from CCl₄ related toxicity studies; CAT, SOD, and GSH decrease levels in liver suggests toxic effect of CCl₄ on the liver (Kumar and Kumar, 2012).

Results show that silymarin is an effective hepatoprotective agent. From the results obtained (table 4.3), it can be concluded that the extract at low dose shows a better hepatoprotection

when compared to the high dose of the extract as demonstrated in liver enzyme level and oxidative stress indicators.

5.3 Histopathological Studies.

5.3.1 Histology of Treated Experimental Animals

Histopathological examination can be used to show the severity of toxicity of CCl₄ induced liver damage (Gnanaprakash *et al.*, 2010). Histoarchitectural distortion manifested as severe alteration in the arrangement of the hepatocytes in the CCl₄ induced group (II), which include areas of inflammatory cells, necrosis, congested central vein, infiltration of inflammatory cells and vacuolar degeneration, (Plate II). All these are signs of toxicity induced by CCl₄. Several researchers had reported on histopathological changes that result from CCl₄ administration, such as necrosis, Kumar and Kumar, 2012; Nevien, 2012).

Mild histoarchitectural distortion of the hepatocytes in the group that received silimarin, a reference drug, (100mg/kg) manifested as mild necrosis, and presence of inflammatory cells when compared with the control group (Plate 3). Necrosis is a pathological type of death that occurs after abnormal stresses, such as chemical injury or toxin. Necrotic cells are unable to maintain membrane integrity; they leak out their content and this may elicit inflammation in the surrounding tissue (Kumar *et al.*, 2009).

Inflammation is fundamentally a protective response, the ultimate goal of which is to get rid of the organism of both the initial cause of the cell injury (e.g., toxins) and the consequences of such injury (e.g., necrotic cells) (Kumar *et al.*, 2009). The administration of standard drug, 100mg/kg Silymarin protected the liver cells against the adverse effects of the toxic agent (CCl₄). The histoarchitecture of this group was close to normal with mild necrosis visible in some areas, but the distortion was not as severe as in control. This is possibly due to the hepatoprotective activity of the drug.

There was observable improvement in the microscopic appearance of the liver after the administration of aqueous extract *Garcinia kola seed* (AEGK) showing restoration in the hepatocytes, mild congestion of the cytoplasm, and absence of centrilobular necrosis with nearly visible central vein, (groups V and VI),(plate 5 and 6). However, the distortion, especially in the group that received 800mg/kg, was not as severe as manifested in the control when compared. The sparing restoration of the hepatocytes in these groups was possibly elicited by the extract treatment due to its hepatoprotective activity. Cellular response to cell injury serves to destroy, dilute, or wall off injurious agents, and it sets into motion a series of events that try to reconstitute the damaged tissue (Kumar *et al.*, 2009).

In the groups administered AEGK, histoarchitectural preservation or protection of the hepatocytes was more effective with 400mg/kg dose. This result is supportive of the biochemical activity studies, see table 4.2. Since *Garcinia kola* is believed to have antioxidant components (Adaramoye, 2005) that can alter the damage done by CCl₄, patients with liver diseases can rely on this naturally available plant as supplement therapy. Therefore one can deduce from these findings that *Garcinia kola seed* tentatively mitigates the effects of CCl₄ on the liver of rats.

5.3.2 Reticular fibres

The structural integrity of the liver is maintained by a delicate meshwork of extracellular matrix in the form of a fine meshwork of reticulin fibres (collage type III).

The reticulin meshwork supports both the hepatocytes and the sinusoidal lining cells (endothelial cells) (Young *et al.*, 2007).

Liver sections from the control group (group I) showed normal histoarchitecture of the liver parenchyma with distinct intensely stained reticular fibres well drawn out from the central vein (Plate 7). This is typical of a normal liver tissue stained with Gordon and Sweets stain. However, liver sections of the CCl₄ treated group (group II) which showed distortion in the arrangement of the liver reticular fibres, (Plate 8) indicate that there was stress on the

hepatocytes from the toxin. This explains why there were breakages to the fibres (Osman *et al*, 2011).

The standard drug (Silymarin) treated group (group III), revealed mild distortion of the reticular fibres, but not as severe as when compared to the CCl₄ treated group (Plate 9). This shows that the drug being a hepatoprotective agent was able to withstand the stress and maintain the integrity of the liver tissue to near normal. This also goes for the extract treated groups, in which the cell was found to have preserved reticular fibres. This activity is probably due to the ability of the extract to protect the liver from the damage caused by CCl₄.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendation

6.1. Summary

The hepatoprotective role of aqueous extract of bitter kola (*Garcinia kola*) seed on CCl₄ induced liver toxicity was conducted using adult Wistar rats.

Aqueous extract of *Garcinia kola seeds* showed hepatoprotection against CCl₄ induced hepatotoxicity through the following mechanisms:

- Aqueous extract of bitter kola seeds showed hepatoprotection on histological sections of the liver in group treated with the extract
- Preservation of the reticular fibres
- Reduction of liver enzyme markers in the serum (ALT, AST and ALP).
- Reduction of oxidative stress through increase in antioxidants (SOD, GSH and CAT).

6.2 Conclusion

In conclusion, medicinal plants are essential source of nutrition and the prevention of human diseases. However, their phytochemicals and action mechanisms are poorly understood. Administration of aqueous seed extract of *Garcinia kola* significantly protected the biochemical and histological alteration to near normal level in liver toxicity-bearing animals and substantially inhibited the liver toxicity incidence in pre-treated groups. From the results it can be inferred that aqueous *Garcinia kola* seed extract (bitter kola) positively modulated antioxidant activity by reducing and detoxifying the free radicals tissue injuries induced by CCl₄. The marked decrease in the levels of lipid peroxides recorded in rats pretreated with *Garcinia kola* seeds suggest that the seed possess the natural antioxidants necessary for protection against free radical damage induced by CCl₄ in rat liver.

Garcinia kola seeds extract showed prophylactic effects on the hepatocytes and reticular fibres of the liver. Its effects in CCl₄ induced liver damage in rats are mediated through anti-oxidation.

From this study it can be concluded that the aqueous extract of the seeds of *Garcinia kola* protects liver against oxidative damages and could be used as an effective protector against CCl₄ induced hepatic damage.

6.3 Recommendation

- Further studies are required to investigate the specific mechanism of action of *Garcinia kola seed*
- Other methods of extraction of the plant (*Garcinia kola*) seeds should also be investigated
- Therapeutic principle involved in hepatoprotection and hepatic gene expression to identify the genes responsible for the protective mechanism.

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