EFFECTS OF AQUEOS EXTRACT OF *CISSUS POPULNEA* ON THE LIVER OF CARBON TETRACHLORIDE TREATRED RATS

BY

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CERTIFICATION

I, DUMNOI FAVOUR EMEKA, hereby certify that this thesis has not been submitted anywhere else in part or in full for any other examination or institution. All literatures and other sources of information consulted, cited or used in this research have been duly acknowledged in references.

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DEDICATION

I dedicate this work to the Almighty God for his infinite goodness, mercies, and wisdom throughout my undergraduate programme. I specially and sincerely dedicate it to my wonderful parents Mr. and Mrs. Benjamin Dumnoi and my siblings Evergreen, Emmanuel Ikechukwu and Esther for their financial support, love and care. May God Almighty bless and replenish them. I also want to thank my friends Isaac, Joyce and Lancelot for moral support, may God bless them.

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ABSTRACT

Cissus populnea has been reported to have high antioxidant content which is beneficial to health. The aim of this study was to investigate the effects aqueous extract of *Cissus pulpolnae* on the liver of Wistar rats. Twenty (20) male Wistar rats were allowed to acclimatize for two weeks under standard laboratory conditions (temperature 24-28°C and 12 hour light-dark cycle) before commencement of the experiment. The rats in each group were allowed access to standard rat chow and water *ad libitum* throughout the experimental period. The rats were randomly assigned into a control group and three treatment groups (5) rats each. The rats in Group A served as control and received feed and water *ad libitum* only. The treatment groups B received intraperitoneal injection of 30% CCl_4 only; group C received 500 mg/kg body weight of aqueous extract of *Cissus populnea* only; group D received 500 mg/kg body weight of aqueous extract of *Cissus populnea* and intraperitoneal injection of 30% CCl_4. The experimental period lasted for 14 days. At the end of the experimental period, the rats were sacrificed under chloroform anaesthesia. Blood samples were collected, in plain bottles, from the Inferior vena cava of each rat for biochemical assay. The liver was excised and fixed in 10% buffered formalsaline for routine histological processing. The data generated were subjected to statistical analysis. Significant difference in the means of all parameters was determined using one way analysis of variance (ANOVA; 95% confidence interval). The result obtained showed that CCL_4 induced some pathologies on the liver tissue ranging from formation of lipid vacuoles (steatosis) to degeneration of the hepatocyte and obliteration of the sinusoids. *Cissus populnea* ameliorated the pathologies induced by CCL_4 on the liver tissue. It is concluded however that *Cissus populnea* possess hepatoprotective potential against CCL_4 insult.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Cissus populnea Guill and Per. (Family: Vitaceae/Amplidacea) is widely distributed in West Africa. Some of its local names include 'Okoho' (Idoma, Igala and Igbo tribes of Nigeria), 'Ogbolo or Ajara' by the Yorubas and 'Dafaaraa or Latutuwa' by the Hausas (Burkill, Plant materials 2000). *C. populnea* is a savannah shrub with a height of about 10 cm and with a diameter of 7.5 cm. It has been linked with numerous therapeutic uses in diverse places. Extracts of *Cissus populnea* have been recognized for antimicrobial properties (Kone *et al.,* 2004), anti-trypanosomal activities (Atawodi *et. al.,* 2002) and anti-sickling properties (Moody *et al.,* 2003). It is used for its diuretic properties in Benin Republic while it is used as post-harvest ethnobotanical protectant in Ghana (Belmain *et al.,* 2000). It is also used to thicken soups, especially among the Idomas of North Central Nigeria. According to previous reviews, the root extract of *C. populnea* has been used for the treatment of wounds, skin diseases and boils (Kone *et al.,* 2004).

It has been reported also to be used in feeding cattle by the fulanis supposedly increasing milk (Ojekale *et al.,* 2006). It is also used in some states in Nigeria for making vegetable soup to stop postnatal bleeding, intestinal parasites and indigestion. It is used as well in the treatment of eye problems that result from attack of black cobra (Soladoye and Chukwuma, 2012). The root cures arrow-poison and also serves as antidote to sore breasts experienced by women at childbearing (Burkill, 2000). The stem bark has been documented to contain flavonoids, carbohydrates, cyanogenic glycosides, tannins, anthraquinones, cardiac glycosides and saponins (Moody *et. al.,* 2003). Infusions of *Cissus populnea* root is popularly sold at car parks and beside the roads to

public transport drivers, including bus and bike riders in South-west Nigeria with the indication that it increases libido. The aim of this study will be to investigate the effects of aqueous extract of *Cissus polpulnae* on the testis of wistar rats treated with doxorubicin.

The testes are two egg-shaped paired organs located between the upper thighs in a skin sack called scrotum. The role of the testes is to secrete the male sex hormone testosterone, which is required for proper physical development in boys; testosterone also maintains libido, muscle strength, and bone density in adults (Schiff *et al.,* 2007). The testis is made up of the tubular compartment (made up of seminiferous tubules, peritubular cells and Sertoli cells) and the interstitial compartment (made up of the Leydig cells, immune cells, nerves, fibroblasts, loose connective tissue, blood and lymph vessels). The seminiferous tubules are the site for spermatogenesis while the interstitial compartment is the site for steroidogenesis. (Weinbauer *et al.,* 2010) Several factors can cause damage to the testis and hinder its main functions of spermatogenesis and steroidogenesis while some elements can also enhance its normal functions, one of these includes herbal medicines.

Doxorubicin is an anthracyline drug first extracted from *Streptomyces peucetius var. caesius* in the 1970's and routinely used in the treatment of several cancers including breast, lung, gastric, ovarian, thyroid, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, sarcoma, and pediatric cancers (Weiss, 1992). There are two proposed mechanisms by which doxorubicin acts in the cancer cell (i) intercalation into DNA and disruption of topoisomerase-II-mediated DNA repair and (ii) generation of free radicals and their damage to cellular membranes, DNA and proteins (Gewirtz, 1999). Doxorubicin is oxidized to semiquinone, an unstable metabolite, which is converted back to doxorubicin in a process that releases reactive oxygen species.

Reactive oxygen species can lead to lipid peroxidation and membrane damage, DNA damage, oxidative stress, and triggers apoptotic pathways of cell death (Doroshow, 1986).

1.2 AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to investigate the effects aqueous extract of *Cissus pulpolnae* on the liver of Rats treated with carbon-tetrachloride. The specific objectives of the study are to investigate the effects of *Cissus pulpolnae* on the;

1. Liver enzymes
2. Antioxidant status of the liver tissue
3. Histology of the liver of rats

CHAPTER TWO

LITERATURE REVIEW

2.1 *CISSUS POLPULNAE*

The genus Cissus consists of about 350 species of which, at least, a dozen is used globally in traditional medicine to treat different ailments. In Australia, Bush Medicine Practitioners use *C. hypoglauca* to treat sore throat (Lassak and McCarthy, 1997). Many cultures in Asia, both East and West Asia, have used locally available species of Cissus to treat several medical problems. In China and the far East, *C. assamica*, is used as anti-snake venom as it decreases endothelin-1 and sarafotoxin 6b (Yang *et al.,* 1998), while in South east Asia including the Indian subcontinent and Sri Lanka, *Cissus quadrangularis* is used for fracture healing (Udupa and Prasad, 1962) and as an anti-obesity agent (Oben *et al.,* 2006). In West Asia, *Cissus hamaderohensis*, is reported to inhibit angiotensin converting enzyme (ACE), neutral endopeptidase (NGP) and aminopeptidase N (APN) (Oleski *et al.,* 2006) as well as have anti-viral properties (Mothana *et al.,* 2006). Several countries in Africa use different species of Cissus in their traditional medicinal practices: Cameroon traditional medicine uses *C. aralioides* as anti-microbial and toxicological agent against microorganisms of the gastrointestinal and urogenital tracts (Assob *et al.,* 2011). Alcoholic extracts of a Gabonese medicinal plant – *C. debilis* - showed antiproliferative activity on human CaCo-2 cells (Line-Edwige *et al.,* 2009). In Nigeria, a few species like *Cissus populnea*, *Cissus ibuensis* and *C. quadrangularis* are used in their native medicine. Methanolic extracts of *C. populnea* increased proliferation of Sertoli cells TM4 in in vitro studies (Osibote *et al.,* 2011) but not in humans treated for 72 days (Ojekale *et al.,* 2006). In addition, it has anti sickling and anti-bacterial properties (Moody *et al.,* 2003; Kone *et al.,* 2004) as well as to treat trypanosomiasis (Atawodi *et al.,* 2002). Most importantly, *C.*

populnea had no adverse side effects after long term administration to Rabbits (Ojekale *et al.,* 2007). *C. ibuensis* is used to treat gastrointestinal problems (Irvine, 1961), rheumatism and arthritis (Dalzeil, 1958). In Congo, *Cissus rubiginosa*, is used as anti-dysentery and antidiarrhoea agent (Otshudi *et al.,* 2000). In the Caribbean islands of Trinidad and Tobago, *C. verticillata* is used as an anti-diabetic agent and to treat urinary problems (Lans, 2006). Moving on to the mainland of South America, in Brazil, *Cissus sycoides* is commonly used as vegetal insulin (Salgado *et al.,* 2009). Of these reports, the most studied are *C. quadrangularis* for obesity, fracture healing and bone diseases and *C. sycoides* as an anti-diabetic agent. We have compiled all the related reports for these two species in this review with an attempt to determine the possibility of using these plants and plant compounds as therapeutic agents to treat or prevent obesity, bone related disease and diabetes.

2.1 CARBON TETRACIILORIDE (CCl_4)

Carbon tetrachloride which is also known as tetrachloromethane is a colourless liquid with a sweet smell (Lewis, 1997). CCl_4 is used experimentally for the induction of liver damage (Parola *et al,* 1992). CCl_4 is a well-established hepatotoxin, previous studies shows that it majorly targets both the liver and the kidney

2.1.1 STRUCTURE AND REACTIVITY

In the Carbon tetrachloride molecule, four chlorine atom are positioned symmetrically as corners in tetrahedral configuration join by single covalent bond. Because of this symmetrical geometry, CCl_4 is non-polar. Methane gas has the same structure, making carbon tetrachloride a

halomethane. As a solvent, it is well suited to dissolving other non-polar compounds, fats and oils. It can also dissolve Iodine.

2.1.2 SYNTHESIS

Carbon tetrachloride can be synthesized in the laboratory by the reaction of chloroform with chlorine but recently CCl_4 is now been synthesized from methane.

$CH_4 + 4Cl_2 — CCl_4 + 4HCl$

The production of CCl_4 from this recent method utilizes by-product of the other chlorination reactions like the synthesis of dichloromethane and chloroform. CCl_4 can also be synthesized by the chlorination of carbon disulfide at 105 to 130°C. $CS_2 + 3Cl_2 \rightarrow CCl_4 + S_2Cl_2$

HISTORY

Carbon tetrachloride was first synthesized in 1839 by a French chemist Henri Victor Regnault by the reaction of chloroform with chlorine. Prior to the late 1950s, carbon tetrachloride was produced primarily by carbon disulfide chlorination (Rossberg, 2002). Immediately after its synthesis by Regnault it was used for a variety of purpose such as a dry-cleaning agent, as a solvent, as a reagent in chemical synthesis, pesticide, grain fumigant, and as a fire extinguisher, National Library of Medicine (Holbrook, 1993, NLM, 2003). However the primary use of CCl_4 was in the production of chlorofluorocarbon (Rossberg, 2002, NLM, 2003). As early as the mid-1970s, annual use and produtction of CCl_4 generally started to decline. The consumer Product Safety Commission banned it use in consumer products in the 1970s. The ban on production and import of carbon tetrachloride in developed countries including the United States, took effect on January 1, 1996. Irrespective of this ban, the manufactured and the import of this chemical for

essential laboratory and analytical uses was still permitted by the United States Environmental Protection Agency (U.S. EPA, 2007).

2.1.4 USES OF CCl_4

2.1.4.1 FIRE EXTINGUSIHER

Carbon tetrachloride was used as a fire extinguisher in early 19th century. A fire extinguisher containing CCl_4 must contain a non-flammable and non-oxidizing gaseous propellant under high pressure. The most likely propellant would be N_2 but a mixture with CO_2 would be quite possible. However, because of its alleged liver toxicity and a human carcinogen, $CC1_4$ fire extinguisher has been placed by bromochlorodifiuoromethane (BCE) ones. The use of BCE fire extinguisher is also in decline as it has been reported that BCF fire extinguishers contains of depleting the ozone-layer, so CO_2 fire extinguishers are widely in use now.

2.1.4.2 DRY CLEANING

In the 1930s, carbon tetrachloride became the first chlorinated solvent employed in dry-cleaning. CCl_4 which is a known central nervous system depressant and a carcinogencan damage both the liver and kidneys on chronic exposure. Due to its high toxicity and corrosive nature, CCl_4 was phased-out as a dry-cleaning solvent in the 1950s.

2.1.4.3 REFRIGERANT

In 1930s-l970s, large quantities of $CC1_4$ were used to produce the chlorofluorocarbon refrigerants R-11 (trichlorofluoromethane) and R-12 (dichlorofluoromethane). Nevertheless, these refrigerants play a role in ozone depletion and as a result their usage has been phased out. Though, Carbon tetrachloride is still used to manufacture less destructive refrigerants.

2.1.5 MECHANISM OF ACTION OF CCl_4

Carbon tetrachloride passes through a particular mechanism to act on the liver and the kidney. CCI_4 is activated by cytochrome (CYP)2E1, CYP2BI or CYP2B2, and possibly CYP3A, to form the trichloromethyl radical, CCI_3. This radical can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration Adduct formation between CCl_3 and DNA is thought to function as initiator of hepatic cancer. This radical can also react with oxygen to form the trichloromethylperoxy radical CCl_3OO^-, a highly reactive species. CCl_3OO^-initiates the chain reaction of lipid peroxidation, which attacks and destroys polyunsaturated fatty acid, in particular those associated with phospholipids. These affect the permeability of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Among the degradation products of fatty acid are reactive aldehydes, especially 4-hydroxynonenal, which bind easily to functional groups of proteins and inhibit important enzyme activities. CCl_4 intoxication also leads to hypomethylation of cellular components. At the molecular level CCl_4 activates tumor necrosis factor (TNF)alpha, nitric oxide (NO), and transforming growth factors (TGF) alpha and beta in the cell, the processes that appear direct the cell primarily toward self-destruction or fibrosis.

2.1.6 ADVERSE EFFECT OF CCl_4

Exposure of the body to CCl_4 at high doses has been reported to have adverse effect on the human body, particularly the liver, kidney and the central nervous system Carbon tetrachloride is rapidly absorbed by any route of exposure in animals. Once absorbed, it is widely distributed

among tissues, especial high lipid content, depending on exposure concentration or dose. It is the liver, lung, and other tissues

2.1.6.1 LIVER DAMAGE

Carbon tetrachloride is metabolized by the liver and it is hepatotoxic. When CCl_4 is ingested into the body it binds with the liver cells (hepatocytes). Low oxygen partial pressure in the liver increases the reductive metabolism of CCl_4 and thus enchances covalent binding. CCI_4 binds to lipids and to proteins throughout subcellular fractions. Binding occurs preferentially to triacylglycerols and phospholipids. The liver cells are inflamed which result in impairment of its functions.

2.1.6.2 CENTRAL NERVOUS SYSTEM DAMAGE

Acute exposure to CCI_4 may lead to rapid central nervous system depression. Carbon tetrachloride rapidly produces a narcotic effect on the brain. In severe conditions, autopsy has revealed several damages to nerve cells (demyelination, purkinje cell damage and patchy pontine necrosis).

2.1.6.3 KIDNEY DAMAGE

Chronic exposure to CCI_4 has been reported to damage the kidney (Ahmed et al., 1999). These damages include; nephritis. In this condition the glomeruli, tubules, or the interstitial tissues surrounding the glomeruli and tubules are inflamed. Another of the effect is proteinuria which refers to the presence of excess serum protein in the urine. The protein in the urine makes it foamy. The major cause of this disorder is the inflammation of the glomerulus or due to low reabsorption at the proximal tubule (Fanconi syndrome).

2.1.7 CARBON TETRACHLORIDE TOXICITY

Carbon tetrachloride is readily absorbed by any route of exposure either orally, intraperitoneally, dermal exposure or by inhalation in animals and humans. Once absorbed by the body, it is widely distributed among tissues, especially those high lipid content. It can cause damage either rapidly or slowly depending on exposure concentration or dose. It is metabolized by the liver, kidney and lung. CCl_4 is rapidly excreted, primarily in exhaled breath. Before the ban of CCI_4 in consumer products in 1970s by the Consumer Product Safety Commission it was a common use of poisoning worldwide.

2.2 SILYMARIN

Silymarin is a flavonolignan which is gotten majorly from the seeds of the milk (*Silyburn marianum*) plant. Silymarin is a polyphenolic flavonoid, extracted using 95% ethanol, from the seeds of the milk thistle. The plant consists of approximately 70-80% of the silymarinflavonoligans and approximately 20-30% of a chemically undefined fraction, comprising mostly polymeric and oxidized polyphenoic compounds (nitin et al., 2007). It is a complex mixture of four flavonolignan isomers, silydianin silybin, isosilybin and silychristin which act as anti-oxidant that neutralizes and break down free radicals (Pradhan and Girish, 2006). Silybin component is reported to be the most active of this three. Milk thistle plant and silymarin are often used interchangebly, but there is a difference between the two. Silymarin is just one of the products of the milk thistle plant. Silymarin, as other flavonoids, has been shown to inhibit P-glycoprotein mediated cellular efflux (Zhou *et aI.,* 2004). The plant has been used medicinally for centuries for the treatment of liver-related disorders and as a result it is widely prescribed by herbalist, it has also been reported to be effective on certain cancers (Nitin *et al.,*

2007). Its common name is derived from the leaves which are characterized by milky veins and its milky white sap, which is traditionally used by nursing mothers to increase milk secretion. This plant was by the English herbalist Culpepper to treat jaundice, gallstones and to cleanse the liver and spleen (Presser *et al.,* 2000).

As reported by Enogieru et al in 2015, silymarin can be used to regenerate liver cells which were damaged by alcohol or drugs. The compound also protects hepatocytes from injury caused by ischaemia, radiation, iron overload and viral hepatitis (Luper *et al.,* 1998). It has also been reported to provide protection against CCl_4 toxicity in the liver and kidney (Mourelle *et al.,* 1989). Silybum marianum is used in traditional chinese medicine to clear heat and relieve toxic material, to soothe the liver and to promote bile flow (Wang *et al.,* 2014).

Silibinin which is also known as silybin is the most active component of a complex of flavonoids of silymarin contained in fruit milk thistle (Sylibum marianum). Its mechanism of action is complex and highly beneficial in protecting hepatocytes. Silibinin itself is mixture of two diastereomers, silybin A and silybin B, in approximately equimolar ratio (Davis-Searles et al., 2005). Research has shown that silybin has hepatoprotective properties, it blocks the penetration of various toxins (for example amanitin) into the hepatocytes thereby preventing cell death, it also prevents apoptosis (Jayaraj et aI., 2007, Al-Anat et aI., 2009).

2.4.1 **ANATOMY OF THE LIVER**

The liver is a reddish brown organ and it is both the largest internal organ and the largest gland in the human body. It has four lobes of unequal size and shape. The human liver weighs about 1500g and weighs about 2%of total body mass of adult (Contran et al., 2005).Anatomically, based only on external features, the liver is described as having four lobes: right, left, caudate, and quadrate lobe. However, functionally, in terms of blood supply and glandular secretion, the

liver is divided into independent right and left lobes. The anatomical large right lobe is separated from the smaller left lobe by the falciform ligament and the left sagittal fissure. The liver is majorly under cover of the rib cage and lies to the right of the stomach and overlies the gall bladder. It is connected totwo large blood vessels; the hepatic artery and the portal vein. Most of the total blood influx is provided by the portal vein bringing nutrient-rich blood from the digestive tract while the hepatic artery delivers blood supplemented with oxygen. These blood vessels subdivided into capillaries which then lead to a lobule. Each lobule is made up of millions of hepatic cells which are the basic metabolic cells. It has two surfaces; diaphragmatic and visceral. The diaphragmatic surface is boldly convex, moulded to the under surface of the

2.4.2 DEVELOPMENT OF THE LIVER

The hepatic diverticulum is seen at the 18th day of gestation (2.5mm stage) as a thickening of the ventral floor of the distal foregut endoderm. This small hepatic diverticulum is the analog for the development of the liver, extrahepatic biliary ducts, gallbladder, and ventral pancreas. Dynamic signaling plays a role for the specification (second stage) of embryonic liver progenitors. Bone morphogcnetic protein from septum transversum, transforming growth factor-beta (TGF beta), and fibroblast growth factor signaling pathways from hepato-cardiac mesoderm converge on the earliest genes that elicit pancreas and liver induction in mouse embryos. The above signaling factors specify the ventral foregut entoderm to become a precursor of hepatic epithelium by expressing several liver-specific genes. The hepatic diverticulum then divides into a solid cranial portion and a hollow caudal one, the cystic part. The cranial part forms the hepatic parenchyma, and differentiates into proliferating cords of hepatocytes and intrahepatic bile ducts, while the smaller cystic portion is the primordium of the gall bladder, common bile duct and cystic duct.

The parenchymal cords anastomose around pre-existing endothelial-lined spaces. They increase in mass and become more organized (Morphogenesis stage) at the expense of the septum transversum that eventually forms the liver capsule. Primitive hepatocytes in contact with the mesenchyme surrounding developing hepatic portal veins form a single structure known as the ductal plate. The ductal plate becomes bilayered with parenchymal and a mesenchymal facing sheet, respectively. The ductal plate consists of cuboidal cells with increased immunoreactivity for epithelial intermediate filaments such as cyto-keratins relative to the surrounding parenchymal cells. The ductal plate gives rise to cholangiocytes lining the intrahepatic bile ducts, including its most proximal segment. It also generates periportal hepatocytes and adult hepatic progenitor cells. The budding liver invades the viteline veins and then the umbilical veins. Vitelline veins run from gut-yolk sac to the heart. The cranial ends of the veins persist as the portal vein and the cadual ends as the hepatic veins. The hepatocytes grow as thick epithelial plates intermingling branches of vitelline veins within the septum transversum to form a system of connecting liver cells plates. On the other hand, the angioblast forms the liver sinusoids. These sinusoids present by the 5th week of gestation act as templates for the three dimensional growth of hepatic cords. Initially, liver cell plates are 3 to 5 cell thick. Then gradually they become one cell thick plates. Intrahepatic bile ducts begin to form at 6th week of gestation at the hilum of the liver and gradually reach the periphery at 3 months. By the 5th week, all elements of the biliary tree are recognizable. Marked elongation of' the common duct occurs with plugging of the lumen by epithelial cells. Recanalization of the lumen of the common duct starts at the end of the 5th week and moves slowly distally. By the 6th week, the common duct and ventral pancreatic bud rotate 180 degrees clockwise around the duodenum. Early in the 7th week, the bile and pancreatic ducts end in closed cavities of the duodenum. Notch signaling is required for normal

duct formation. That means it stimulates the cells adjacent to the hepatocyte to differentiate another cell type (duct cells). Notch signals are required for bile duct morphogenesis and activation of Notch signaling in the hepatic lobule promotes on and tubule formation in a dose-dependent manner. The originally hollow cystic portion becomes obliterated owing to the rapid proliferation of its epithelium. At first the gall bladder and common bile duct are solid cords under the developing liver in the 6 to 7mm embryo. Recanalization of the hepatic, common bile duct, cystic duct, and proximal gall bladder then occur by the 16mm embryo. At the third month, the gall bladder is fully open, and connected with the intrahepatic biliary system.

2.4.3 MOLECULAR REGULATION OF THE LIVER INDUCTION

The foregut endoderm has the potential to express liver-specific genes and to differentiate into liver tissue. However, this expression is blocked by factors produced surrounding tissues, including ectoderm noncardiac mesoderm and particularly e notochord The action of these inhibitors is blocked in the prospective hepatic region by fibroblastgrowth factors (FGF2)secreted by cardiac mesoderm and by blood vessel-forming endothelial cells adjacent to the gut tube at the site of liver bud outgrowth Thus, the cardiac mesoderm together with neighbouring vascular endothelial cells "instructs" gut endoderm to express liver-specific genes by inhibiting an inhibitory factor of these same genes. Once this "instruction" is received, cells in the liver field differentiate into hepatoces and biliary cell lineages, a process that is at least partially regulated by hepatocyte nuclear transcription factors (HNF3 and 4) (Sadler.,2012).

2.4.4 ABNORMALITIES

Liver abnormalities are conditions that affect the functions or structure of the liver Abnormalities of the liver could be morphological, vascular, and hereditary.

2.4.4.1 MORPFIOLOGICAL ANOMALIES

Morphological developmental anomalies include: agenesis (absence of a lobe that is replaced by fibrous tissue); aplasia (small lobe with abnormal structure, few hepatic trabeculae, numerous bile ducts, and abnormal blood vessels); and hypoplasia (small lobe but with normal structure). Agenesis of the right lobe of the liver is a rare finding with preservation of the middle hepatic vein. It is usually an incident finding revealed by imaging exams or during abdominal surgery. Hvpoplasia of right hepatic lobe is a rare congenital anomaly that is sometimes associated with ectopy of gall bladder.

2.4.4.2 VASCULAR ANOMALIES

Variation in hepatic arterial anatomy is seen in 40-45% of people. Classic branching of the common hepatic artery from the celiac artery, and the proper hepatic artery into right and left hepatic arteries to supply the entire liver, is seen in 5 5-60%. In general, the common hepatic artery may arise from the abdominal aorta or superior mesenteric artery (SMA), and all or part of the right and left hepatic arteries may arise from other vessels. The two commonest variants are right hepatic artery replaced to the SMA and left hepatic artery replaced to the left gastric artery.

2.4.4.3 HEREDITARY ANOMALIES

Hereditary hemorrhagic telangiectasia (T, Osier-Weber-Rendu syndrorme), is an autosomal dominant vascular disorder with a variety of clinical manifestations (epistaxis, gastrointestinal

bleeding, characteristic mucocutaneous telangiectasia). In addition, arteriovenous malformations (AVMs) commonly occur in the pulmonary, hepatic, and cerebral circulations. Large AVMs between the hepatic artery and hepatic vein can cause a significant left-to-right shunt with increased cardiac output (Garcia Tsao et al., 2000). Portal hypertension and hepatic encephalopathy, particularly after episodes of gastrointestinal bleeding, may result both from shunts between the hepatic artery and portal vein, and from increased sinusoidal blood how, leading to enhanced deposition of fibrous tissue and cirrhosis of the liver.

Ataxia-telangiectasia is an autosomal recessive, multisystem disorder characterized by progressive neurologic impairment, variable immunodeficiency with susceptibility to sinusitis and pulmonary infections, impaired organ maturation, x-ray hypersensitivity, ocular and cutaneous telangiectasia, and a predisposition to malignancy. Veno-occlusive disease of the liver may accompany ataxia telangiectasia.

Hippel-Lundau disease is a rare autosomal dominant familial tumor syndrome associated with brain, retinal, and spinal cord hemangioblastoma; renal cysts and renal cell carcinoma; pheochromocytoma; and pancreatic cysts, pancreatic serous cystadenomas, and pancreatic neuroendocrine tumors. Liver cysts have been associated with von Hippel-Lindau disease.

2.4.5 DISEASES OF THE LIVER

The liver is a vital organ in the body that supports nearly every other organ in the body in some facet. Without a healthy liver a person cannot 9inive. Common liver diseases include hepatitis A, B, C, D, E, fatty liver disease, cancer, cirrhosis damage from alcohol, the pain reliever acetaminophen, and other cancer drugs. Most times, liver diseases are accompanied by jaundice which is as a result of increased level of bilirubin in the system. The bilirubin accumulates as a

result of the breaking down of' haemoglobin which is gotten from the haemolysis of red blood cells. It is the function of the liver to remove bilirubin from the blood and excrete it through bile, but in diseased condition, this function is impaired. Liver diseases may be diagnosed by a liver function test. One advantage of the liver is its ability to regenerate.

CHAPTER THREE

MATERIALS AND METHODS

3.1 ANIMAL CARE AND MANAGEMENT:

Twenty (20) Wistar rats would be selected from the animal holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city . The animals in each group would be allowed access to 300g / day Top feed Growers ' mash, manufactured by Premier feed mills co .Itd (a subsidiary of flour mills of Nigeria Plc.) and water *ad libitum.*

3.2 TREATMENT PLAN

Animals in group A would serve as control; group B rats were treated with CCL_4 only; group C rats were treated with 500mg/kg body weight of *Cissus populnea* only; group D rats were treated with 500mg/kg body weight of *Cissus populnea* and CCL_4. The experimental period will last for 28 days.

3.3 SACRIFICE OF ANIMALS AND SURGICAL REMOVAL OF TISSUES

At the end of the experiment period, the animals were grossly observed for general physical characteristics, mobility and agility. They were also screened for the presence of opened wounds. The animals were weighed using a top loader weighing balance. A mid-line incision was made through the anterior abdominal walls of the rats under slight anesthesia using chloroform. The liver was excised, weighed and fixed in 10% buffered formal saline fixative for 24 hours.

3.4 TISSUE PROCESSING

The liver was fixed in 10% buffered formal saline for 48 hours. The times were then trimmed to about 3-5mm thick sections and processed via paraffin wax bathing method of Drury and Wallington (1980). The tissues were dehydrated for one hour each at room temperature through ascending grades of ethanol: 70 % ethanol, 90 % than Absolute ethanol 1. Absolute ethanol II.

Dehydrated issues were cleared at rooms' temperature in two changes of xylene for one hour in cache change. The tissues were then infiltrated in two changes of molten paraffin wax at 60 ° C for one hour in each change and finally embedded in paraffin wax multi - block plastic embedding molds. The rotary microtome. Paraffin blocked tissues were trimmed and mounted on wooden block for sectioning on a rotary microtome.

Sections of 5um thickness were produced from the tissue blocks using a rotary microtome (Bright B5143, Huntington, England) . The sections were transferred into water bath (40 °C) to allow spreading of the folded ribbons of sections. These sections would be mounted on new clean glass slides. These were dried at 40 °C on a slide drier to enhance adherence of the sections of the slides.

3.5 HEMATOXYLINE AND EOSINE STAINING PROCEDURES

Paraffin wax is poorly permeable to stain; so section were then deparafinized in two changes of xylene for two minutes in each change . Xylene were removed because it does not mix freely with aqueous solution and low grades of alcohol used in preparing stams. Thus , sections were passed through two changes of absolute alcohol for four minutes each . The sections were hydrated using a series of descending grades of alcohol until water is used . The purpose of this process is to prepare the tissue to stain with a dye that would have been dissolved in an aqueous

solvent. Procedures of H and E as adopted as follows : described by Drury and Wallington (1980) and Scheehan and Hrapchak (1980) would be adopted as follows:

a . Sections were dewaxed in two changes of xylene for two minutes in each change ;

b . They were rehydrated in descending grades of alcohol (absolute 1 , absolute II , 95 % , 90 % , 70 % and 50 % ethanol) for two minutes each ; c . The sections were rinsed in distilled water for three minutes ;

d . The sections were stained in hematoxylin for 15-20 minutes ;

e. Excess hematoxylin stain would be removed by rinsing well in running tap water for two to three minutes (sections were examined microscopically at this stage to confirm sufficient degree of staining) ;

f . Sections were differentiated in acid alcohol (0.5% HCL in 70% ethanol) for two to three seconds. The blue staining of hematoxylin was changed to red by the action of the acid ;

g . Sections were rinsed well in running water for 10-15 minutes to remove excess differentiator and regain the blue color of the sections as observed with naked eye ;

h . Sections were counterstained in 1 % aqueous eosin for two to four minutes ;

i . Excess stain were washed off in running tap water and examined under microscope.

j . Sections were dehydrated rapidly in ascending grades of ethanol (50 % through absolute ethanol) , cleared in xylene and mounted in a synthetic resin medium (DPX) using clean glass cover slip.

3.6 PHOTOMICROGRAPHY

The sections were examined under Leica DM750 research microscope with a digital cainera Leica ICC50) attached. Digital photomicrographs of the tissue sections were taken at various magnifications.

3.7 STATISTICAL ANALYSIS

The data generated were analyzed using descriptive and inferential statistics. All values would be presented as mean ± Standard Error of Means (S.E.M.). All statistical analysis were carried out using Statistical Package for Social Sciences (SPSS) (version 17). The significance of difference in the means of all parameters would be determined using one way analysis of variance (ANOVA; 95% confidence interval). Least Square Difference (LSD), post hoc test were carried out for all groups with control and comparison of all pairs of groups respectively. Statistical significance were considered significant at $P < 0.05$.

CHAPTER FOUR

RESULT

4.1 LIVER FUNCTION ENZYMES

Table 4.1 shows the result obtained from the liver function test (Aspartate amino transferase (AST), Alkaline phosphatase (ALP), Alanin transaminase (ALT) and Total bilirubin (TB). Analysis of the data showed that there was a significant (P>0.05) increase in serum AST, ALT, ALP and T.Bil levels of rats treated with CCL_4 only compared with control. There was no significant (P<0.05) difference in the levels of AST, ALT, ALP and T.Bil in the group treated with *Cissus populnea* only and those co-treated with *Cissus populnea* and CCL_4.

4.2 ANTIOXIDANT ENZYMES

Table 4.2 shows the result obtained from tissue antioxidant enzymes and lipid peroxidation (SOD, CAT, GPx and MDA respectively). There was a significant (P>0.05) decrease in level of SOD, CAT, GPx and increase in the level of MDA of rats treated with CCL_4 only compared with control. There was no significant (P<0.05) difference in the levels of SOD, CAT, GPx and MDA in the liver tissue homogenates of rats in the group treated with *Cissus populnea* only and those co-treated with *Cissus populnea* and CCL_4.

4.3 HISTOLOGY

Plate 4.1-plate 4.14 represent the histological result. Liver slides from control group showed normal liver histoarchitecture; central vein, hepatocytes, sinusoids and the portal area. The slide of rats treated with CCL4 only showed histological distortions; fatty impregnated vacuoles (Steatosis) and hepatocyte degeneration

Table 4.1: Liver enzyme and Bilirubin profile of rats treated with *Cissus populnea* and CCL_4

Grouping	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**T.BIL (mg/dl)**
	n=5	n=5	n=5	n=5
Group A (control)	80.000 ± 6.000	23.532 ± 2.410	30.210 ± 2.018	0.164 ± 0.021
Group B (CCL_4 only)	134.300 ± 3.220*	101.330 ± 4.30*	69.021 ± 2.309*	0.843 ± 0.030*
Group C (*Cissus populnea* and CCL_4)	76.250 ± 5.320	33.330 ± 3.324	35.330 ± 3.194	0.122 ± 0.031
Group D (*Cissus populnea* and CCL_4)	82.300 ± 6.333	24.330 ± 2.540	27.200 ± 1.309	0.120 ± 0.030

Data is represented as Mean ± SEM; *Superscript represents significant difference of means of treatment groups compared with control at $P<0.05$.

Table 4.2: Anti-oxidant profile of rats treated with extract of *Cissus populnea* and CCL_4

Grouping	**SOD (U/g)**	**CAT (U/g)**	**GPx (U/g)**	**MDA (mol/g)**
	n=5	n=5	n=5	n=5
Group A (Control)	0.620 ± 0.021	0.148 ± 0.001	1.347 ± 0.022	0.332 ± 0.012
Group B (CCL_4 only)	0.113 ± 0.013*	0.033 ± 0.003*	0.469 ± 0.013*	0.931 ± 0.021*
Group C (*Cissus populnea* only)	0.596 ± 0.033	0.162 ± 0.006	1.253 ± 0.016	0.320 ± 0.013
Group D (*Cissus populnea* and CCL_4)	0.584 ± 0.016	0.151 ± 0.001	1.322 ± 0.017	0.231 ± 0.030

Data is represented as Mean ± SEM; *Superscript represents significant difference of means of treatment groups compared with control at $P<0.05$.

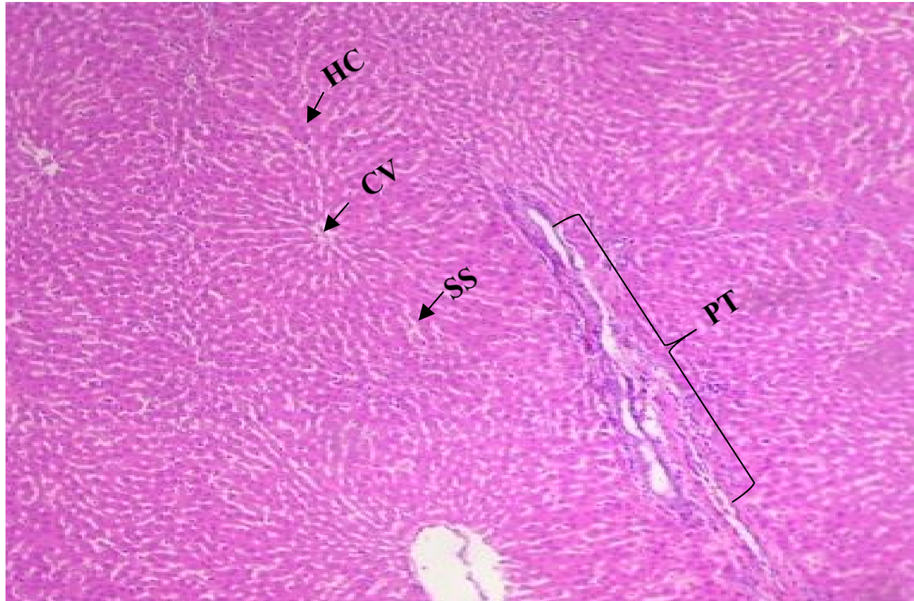

Plate 4.1: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC), Portal area (PT), Central vein (CV), sinusoids (SS) H & E x 40

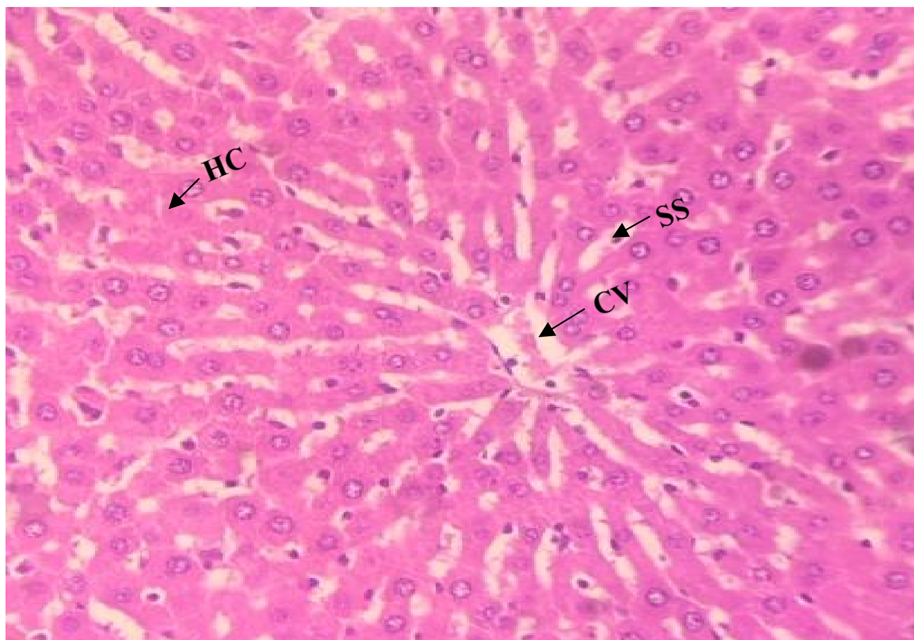

Plate 4.2: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC, Central vein (CV), sinusoids (SS) H & E x 100

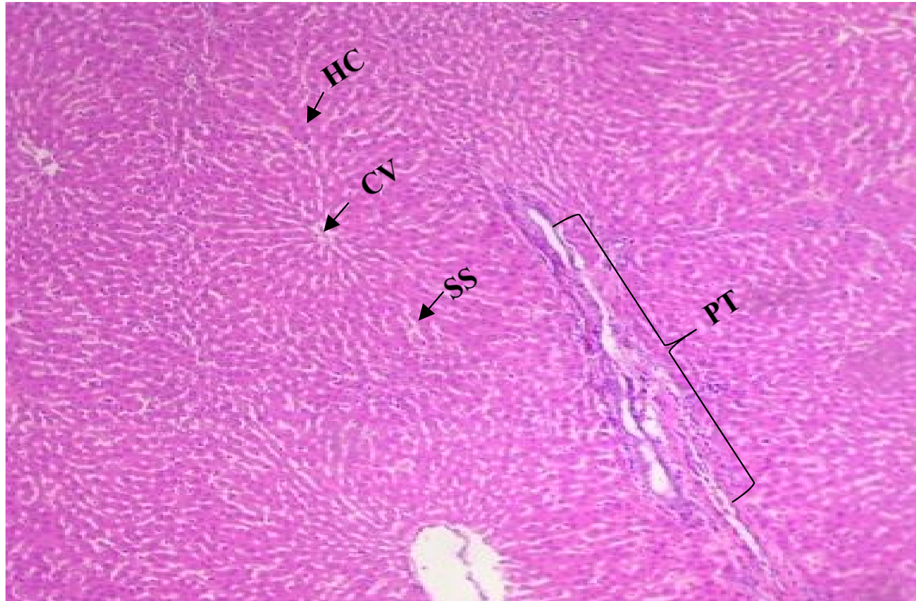

Plate 4.3: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC), Portal area (PT), Central vein (CV), sinusoids (SS) H & E x 40

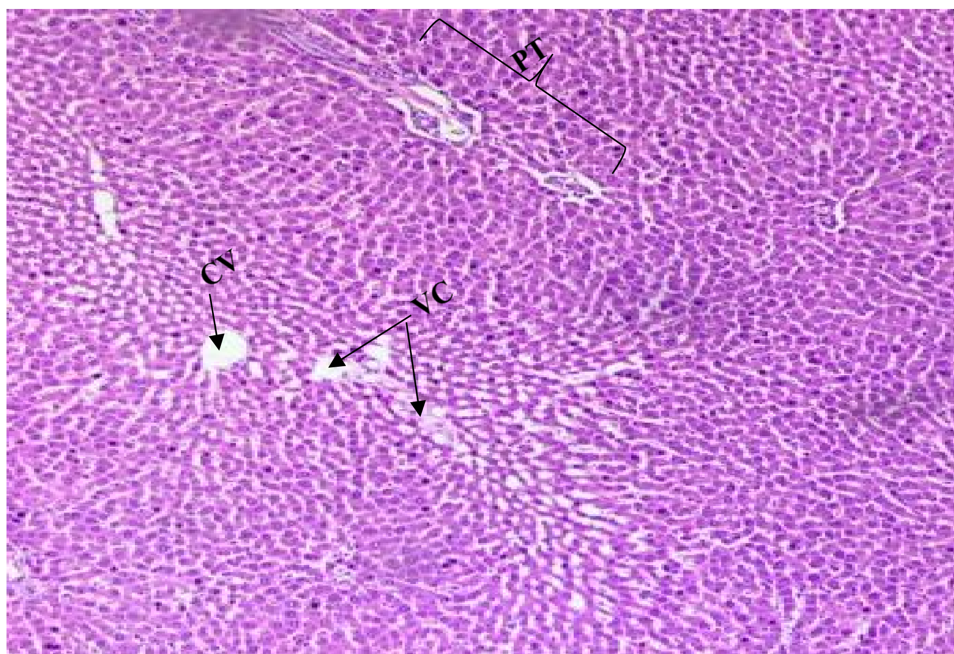

Plate 4.4: Micrograph of liver section of rats in the group (B) treated with CCL_4: features seen in section include; Portal area (PT), Central vein (CV), Vacuoles (VC), Hepatocyte degeneration (HD) H & E x 40

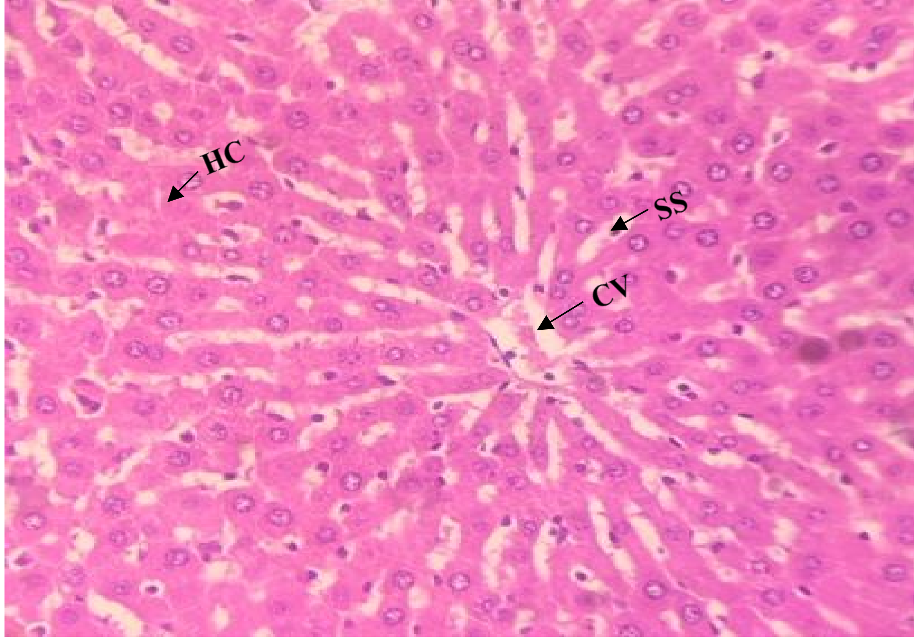

Plate 4.5: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC, Central vein (CV), sinusoids (SS) H & E x 100

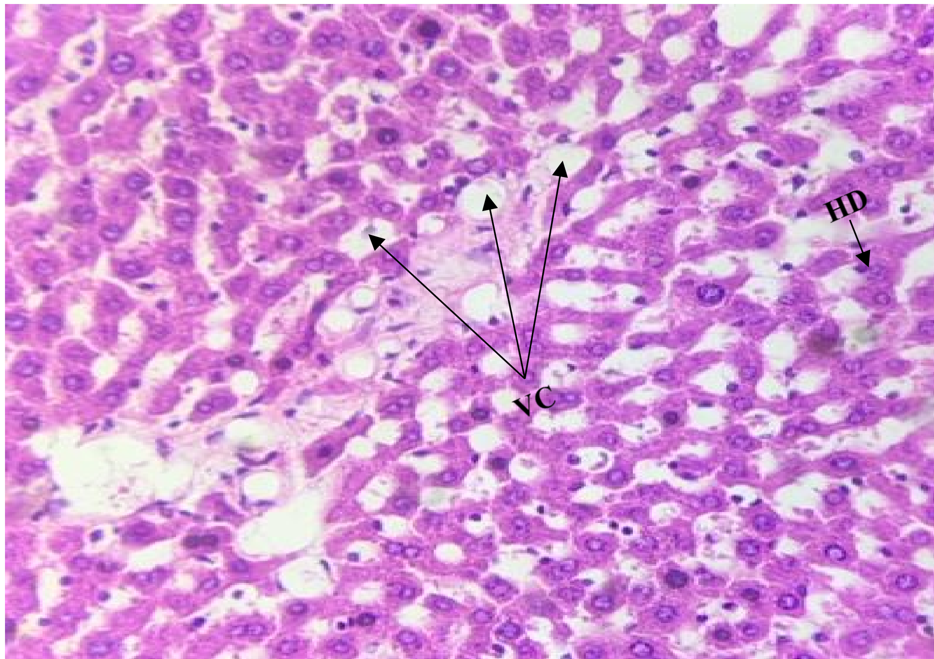

Plate 4.6: Micrograph of liver section of rats in the group (B) treated with CCL_4: features seen in section include; Vacuoles (VC), Hepatocyte degeneration (HD) H & E x 100

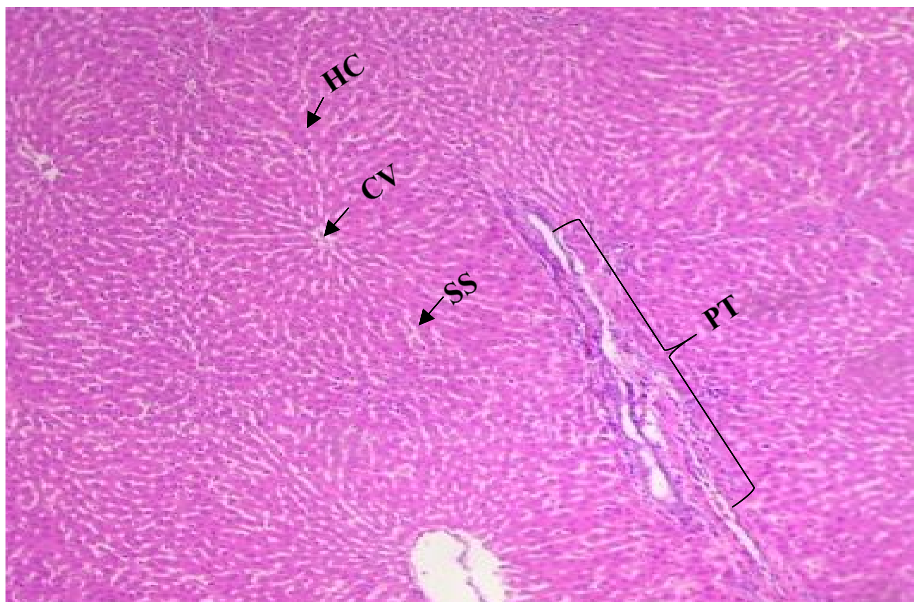

Plate 4.7: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC), Portal area (PT), Central vein (CV), sinusoids (SS) H & E x 40

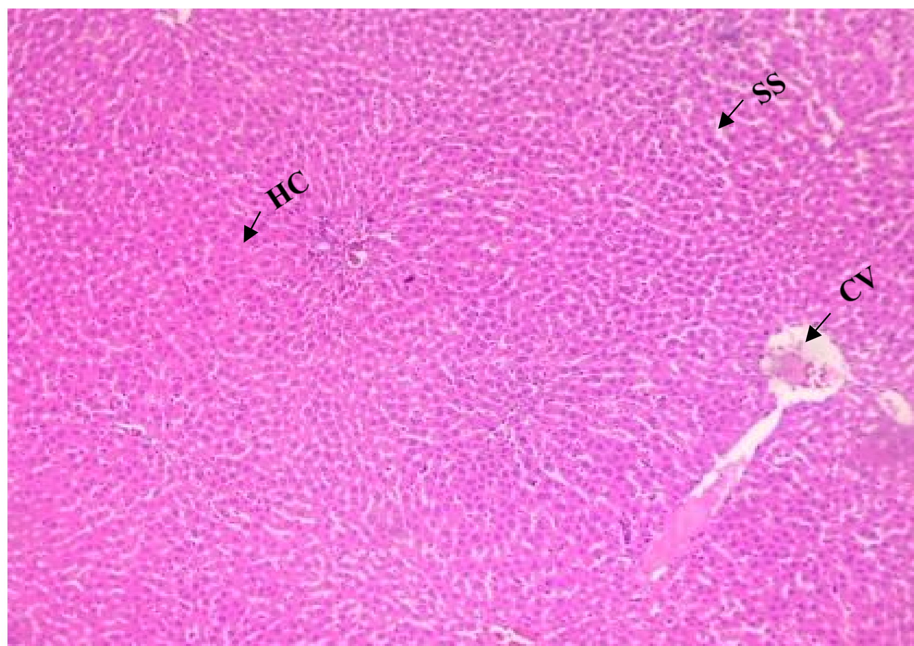

Plate 4.8: Micrograph of liver section of rats in the group (C) treated with *Cissus populnae* extract: features seen in section include; hepatocytes (HC), Central vein (CV), sinusoids (SS) H & E x 40

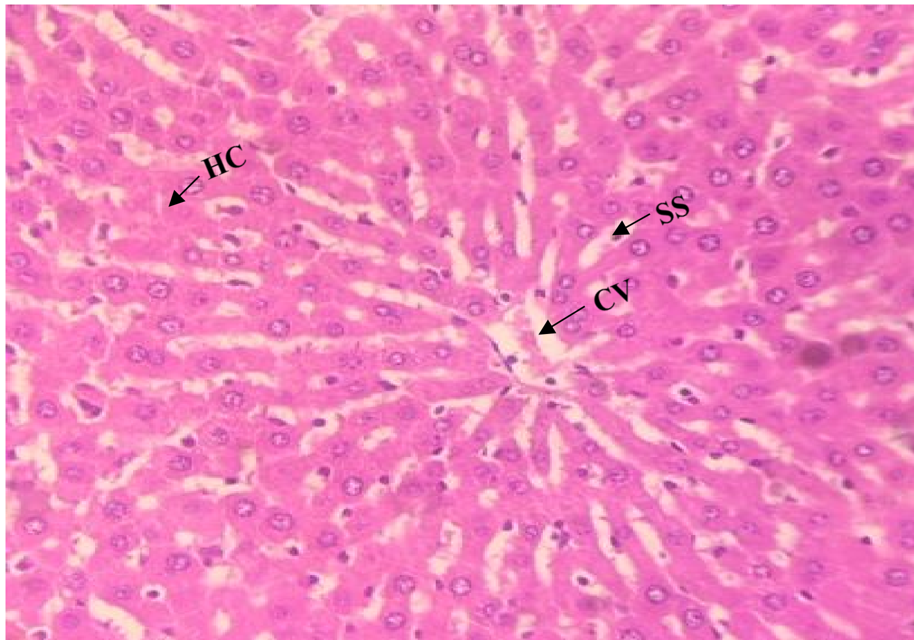

Plate 4.9: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC, Central vein (CV), sinusoids (SS) H & E x 100

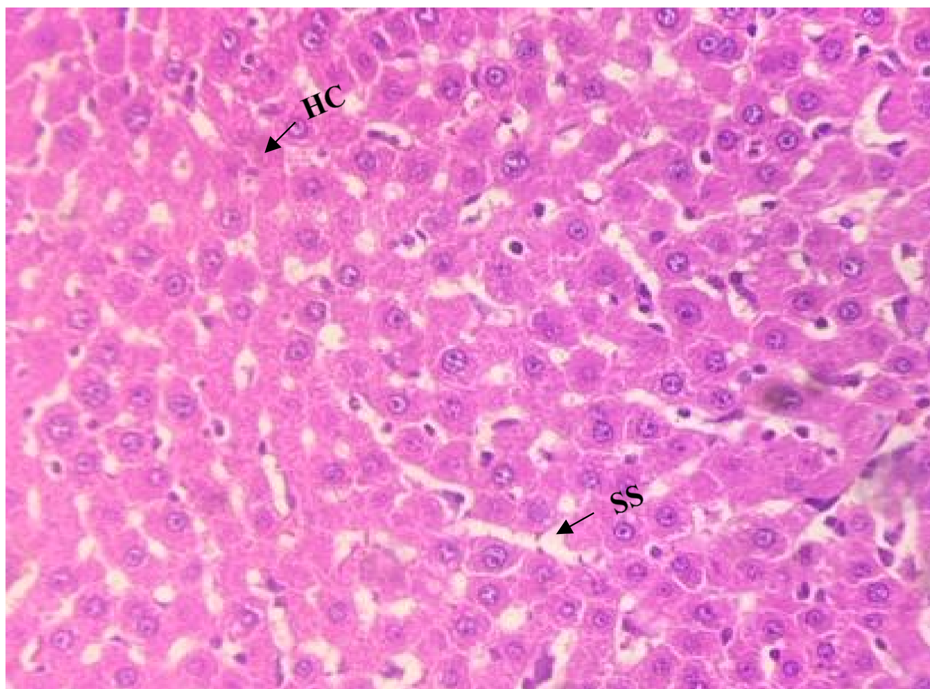

Plate 4.10: Micrograph of liver section of rats in the group (C) treated with *Cissus populnae* extract: features seen in section include; hepatocytes (HC), sinusoids (SS) H & E x 100

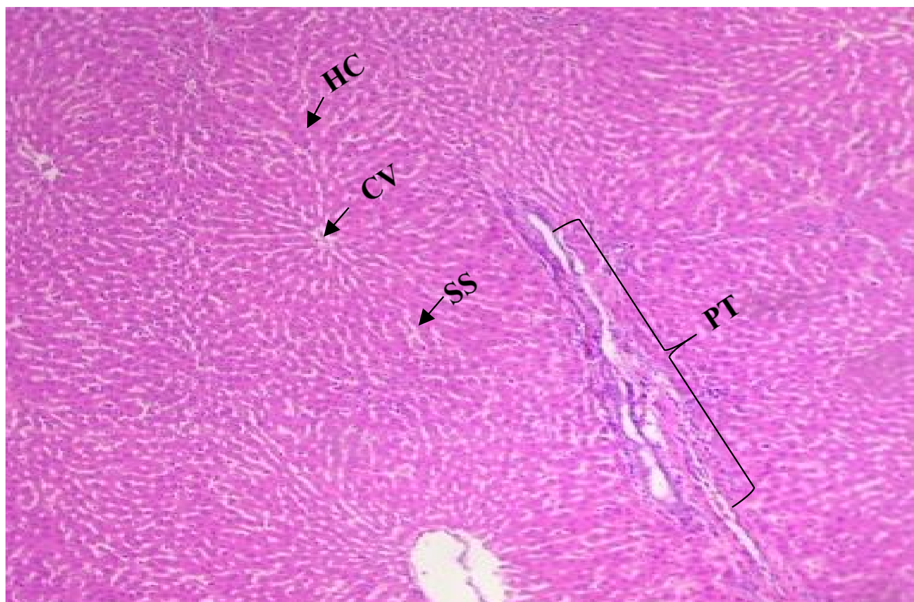

Plate 4.11: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC), Portal area (PT), Central vein (CV), sinusoids (SS) H & E x 40

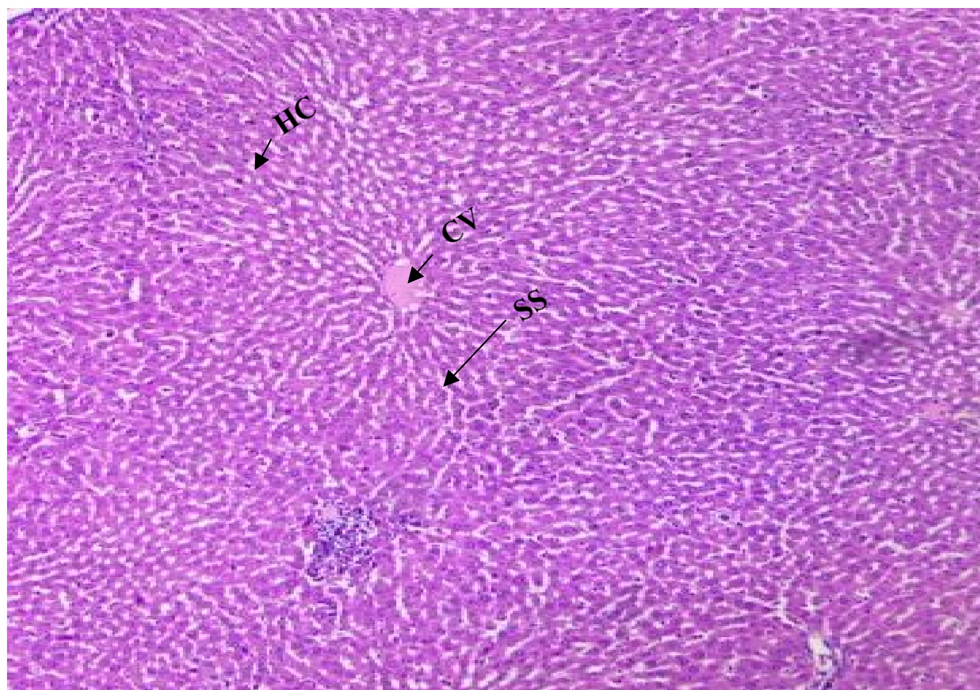

Plate 4.12: Micrograph of liver section of rats in the group (C) treated with *Cissus populnae* and CCL_4 extract: features seen in section include; hepatocytes (HC), Central vein (CV), sinusoids (SS) H & E x 40

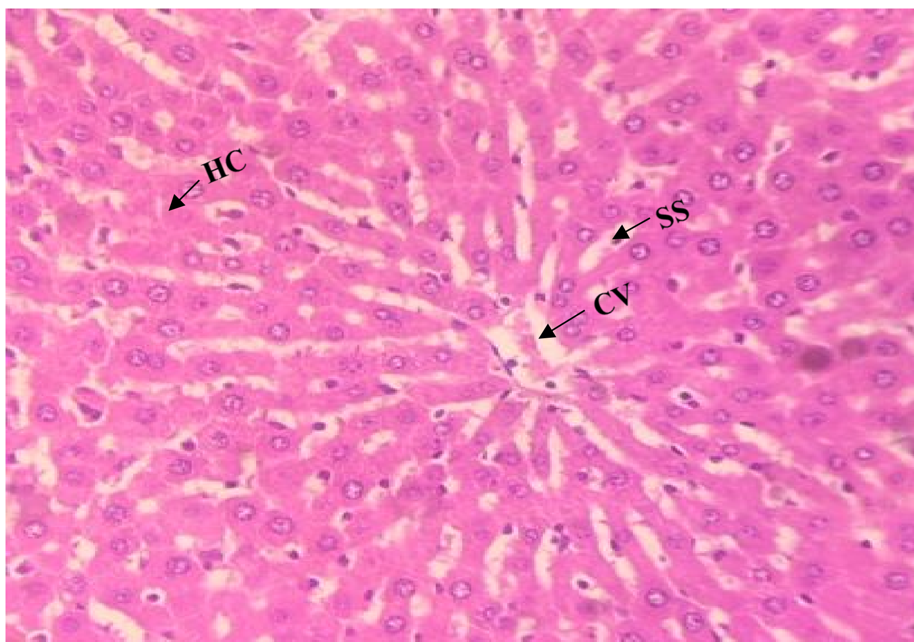

Plate 4.13: Micrograph of liver section of rats in the control group (A): features seen in section include; hepatocytes (HC, Central vein (CV), sinusoids (SS) H & E x 100

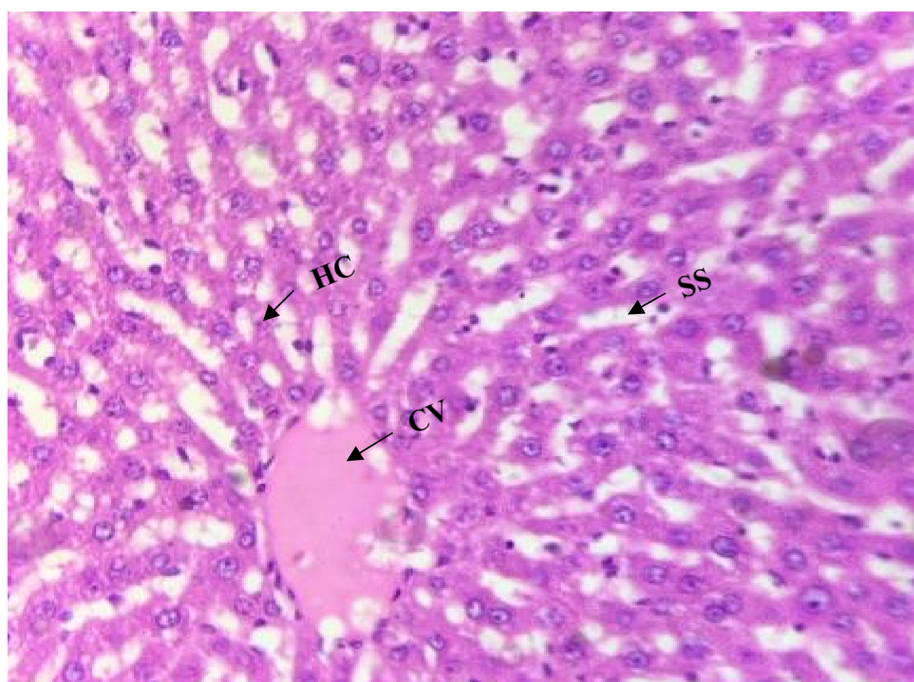

Plate 4.14: Micrograph of liver section of rats in the group (C) treated with *Cissus populnae* and CCL_4 extract: features seen in section include; hepatocytes (HC), Central vein (CV), sinusoids (SS) H & E x 100

CHAPTER FIVE

DISCUSSION

The liver is centre of metabolism and detoxification of drugs or chemical substances introduced into the body system and thus is faced with great risk of damage. Carbontetrachloride is a known hepatotoxic agent that is commonly deposited in the liver. The liver functional transaminases (AST and ALT) and alkaline phosphatase (ALP) enzymes activity in serum are most frequently measured for diagnosis of liver diseases particularly infective hepatitis, alcoholic cirrhosis, biliary obstruction, toxic hepatitis and liver cancer (Zaahkouk *et al*., 2000 and Abdel-Wahab *et al*., 2007). AST, ALP and ALT liver enzymes are not secreted into the blood; any elevation of their activities in blood is resulted from leakage of liver damage cells and from the disturbance and dysfunctions in liver functional enzymes (Abu-Zeid, 2001 and Attia and Nasr, 2009). In this study, group B (treated with 50mg/kg body weight of CCL_4 only) caused a significant increase ($P<0.05$) in aminotransferases and alkaline phosphatase enzymes in blood serum when compared to control. This is in agreement with previous study which reported that lead has hepatotoxic effects as it increases liver enzymes level in blood serum (Nabil *et al., 2011* and Abdou *et al.,* 2007). There was however a significant reversal and restoration of these enzyme levels in group C (treated with 500mg/kg body weight of *Cissus populnea*) and group D (treated with 500mg/kg body weight of *Cissus populnea* and CCL_4). This is in agreement with the commonly accepted view that serum levels of transaminase and alkaline phosphatase return to normal with the healing of the hepatic parenchyma and the regeneration of hepatocytes (Fraschini *et al*., 2002). Group B (treated with CCL_4 only) also showed significant increase ($P<0.05$) in Total bilirubin levels (Tb) when compared to control. This may be due to the induction of heme oxygenase which converts heme to bilirubin thus compromising the entire biliary system of the liver

(Seddik *et al.,* 2010). Histological examination showed liver tissue characterized by patchy hepatocytes degeneration, with microvesicular steatosis in the tissue of rats treated with lead acetate only. This further support the results obtained from the liver function test assay, where liver enzymes level was significantly elevated in the group treated with lead acetate only. Rats treated with aqueous leave extract of *Cissus populnea* showed histological examination of liver tissue characterized with normal hepatocytes, normal portal microarchitecture. Histological examination also showed normal cyto-architecture of the liver tissue in groups treated with aqueous leave extract of *Cissus populnea* and CCL_4.

In conclusion, the aqueous leave extract of *Cissus populnea* provided hepatoprotective effect from CCL_4-induced damage. The possible mechanism responsible for the hepatoprotective effect by the aqueous leave extract of *Cissus populnea* is due to its antioxidant properties, thus aqueous leave extract of *Cissus populnea* can be used in protective treatment of CCL_4- induced liver damage.

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