HISTOLOGICAL ASSESSMENT OF LIVER DEVELOPMENT FOLLOWING INTRAUTERINE EXPOSURE TO CARBON TETRACHLORIDE IN WISTAR DAMS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this research work titled "**HISTOLOGICAL ASSESSMENT OF LIVER DEVELOPMENT FOLLOWING INTRAUTERINE EXPOSURE TO CARBON TETRACHLORIDE IN WISTAR DAMS**" for the award of a degree of Bachelor of Science (B.Sc.) in Anatomy was carried out by **AGHUGHU TESSY OSARUGUE** under the supervision of **DR. V. C. EZEUKO**. All literatures used in this study have been acknowledged and properly referenced.

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EXTERNAL EXAMINER **DATE**

DEDICATION

ACKNOWLEDGEMENT

TABLE OF CONTENTS

ABSTRACT

The liver is the main organ where exogenous and endogenous chemicals are metabolized and finally excreted. As a consequence, hepatocytes are exposed to remarkable concentrations of these chemicals and drugs, which can lead to cell death, hepatoxicity, liver dysfunction, and even organ failure. During fetal development, the liver plays a crucial role in hematopoiesis, metabolism, and detoxification. Any disruptions or abnormalities in liver development can have long-lasting effects on liver function and overall health. Therefore, understanding the impact of Carbon Tetrachloride (CCl_4) exposure on liver histology during fetal development is of significant interest since CCl_4 is well-known for its hepatotoxic effects, causing liver damage, inflammation, fibrosis, and even hepatocellular carcinoma. This study was aimed at assessing the histological development of the liver following intrauterine exposure to CCl_4. In this study, forty (40) adult Wistar rats weighing between 160 g and 180 g were used. The animals were paired overnight at the estrous cycle with sexually active males in the ratio of 2:1. Estrous cycle was confirmed by vaginal lavage. The presence of vaginal plug and/or sperm in the vaginal smear was GD0. The pregnant rats were divided into two groups (A and B) with twenty (20) rats per group. Group A served as Control and was administered with a single intraperitoneal injection of 0.2 ml of normal saline on GD 8. Group B served as the treated group and was administered a mixture of CCl_4 and olive oil: 2 ml/kg body weight intraperitoneally. On GD15, GD17, GD19, and GD21 five (5) animals were sampled from each group. The uterine horns were exteriorized and incised at the greater curvature of the horns. Fetal liver tissues were harvested from each group for histological assessment. Histological results showed that on GD 15, persistent erythroblastic island was evident, suggesting an ongoing compensatory response to hematopoietic disturbances induced by CCl_4. By GD 17, occluded central veins were evident indicative of vascular disruptions within the liver while congested vessels were present on GD 19 indicative of altered vascular architecture and blood flow dynamics. GD 21 showed occlusion of central veins indicating a lasting impact of CCl_4 on the vascular integrity of the fetal liver. In conclusion, this study showed that carbon tetrachloride (CCl_4) has teratogenic potential against liver development in Wistar rats.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The liver and kidneys are the main organs affected by drug and chemical toxicity (Zhang *et al.,* 2007). The liver is the central organ for metabolism and detoxification in the human body, which leaves it vulnerable to various risk factors, like metabolic disorders, alcohol consumption, viral infection, or toxin exposure. The liver is the main organ where exogenous and endogenous chemicals are metabolized and finally excreted (Almazroo *et al.,* 2017). As a consequence, hepatocytes (liver cells) are exposed to remarkable concentrations of these chemicals and drugs, which can lead to cell death, hepatoxicity, liver dysfunction, and even organ failure (Russmann *et al.,* 2009). Therefore, the functional integrity of the mammalian liver is vital to total body homeostasis (Burcham 2014). During fetal development, the liver plays a crucial role in hematopoiesis, metabolism, and detoxification. Any disruptions or abnormalities in liver development can have long-lasting effects on liver function and overall health. Therefore, understanding the impact of CCl_4 exposure on liver histology during fetal development is of significant interest. It is well-known for its hepatotoxic effects, causing liver damage, inflammation, fibrosis, and even hepatocellular carcinoma. A toxic insult to the liver could disrupt any or all of these activities and could have profound effects (Zhang *et al.,* 2012). Therefore, the liver is more commonly involved in overt organ toxicity than most other tissues (Zhang *et al.,* 2012).

Accidental or deliberate toxicity with drugs can cause life-threatening liver damage. Moreover, these medicinal risks and liver injuries accompany exposure to different

chemicals in the environment or workplace. Still, chemicals such as food contaminants that harm these excretory organs are consumed (Burcham, 2014). Therefore, protecting the liver against these agents is inevitable. Several studies have investigated the hepatotoxic effects of CCl_4 in adult animals and have characterized the histological changes associated with CCl_4-induced liver injury. However, there is a paucity of research specifically examining the effects of CCl_4 on liver histology during fetal development.

1.2 AIM AND OBJECTIVES

The study was aimed at assessing the histology of liver development following exposure to Carbon Tetrachloride (CCl_4) in Wistar rat dams.

Specific objectives of the study

The specific objective of this study was to assess the CCl_4 exposure on the histology of the fetal liver of Wistar rat dams.

1.3 STATEMENT OF RESEARCH PROBLEM

Carbon tetrachloride (CCl_4) is a toxic chemical that has been used for many years as a solvent and degreaser. It is also a known hepatotoxin, meaning that it can damage the liver. CCl_4 exposure can cause a variety of liver injuries, including steatosis (fatty liver), necrosis (cell death), and fibrosis (scarring). CCl_4 is a common environmental contaminant, and humans can be exposed to it through inhalation, ingestion, or skin contact. Pregnant women are particularly vulnerable to the effects of CCl4 exposure, as it can cross the placenta and reach the developing fetus.

Studies have shown that CCl_4 exposure during pregnancy can lead to a variety of adverse outcomes for the offspring, including premature birth, low birth weight, and birth defects. CCl4 exposure can also damage the developing liver, which can lead to long-term health problems such as liver disease and cancer. Despite the known risks of CCl4 exposure during pregnancy, there is limited information on the specific effects of CCl4 on liver development. This study aims to assess the histology of liver development in Wistar rat dams following exposure to CCl4.

1.4 SIGNIFICANCE OF THE STUDY

While the hepatotoxic effects of CCl_4 in adult animals have been extensively studied, limited research has focused on its effects on liver development during fetal stages. Investigating CCl_4's impact on liver histology throughout fetal development offers crucial information about the chemical's potential developmental toxicity. Disruptions in the development of the liver, which is essential for many metabolic functions including detoxification, may have long-term effects on liver health and function. Examining the histological changes in the liver following CCl_4 exposure helps to understand the underlying mechanisms involved in liver development and how they may be disrupted by toxicants. This knowledge can contribute to a better understanding of normal liver development and aid in the identification of key cellular and molecular targets affected by CCl_4 exposure. Insights into potential dangers and processes of liver development disruption in humans exposed to CCl_4 or comparable toxic compounds can be gained by understanding the histological changes in the liver

brought on by CCl_4 exposure in Wistar rat dams. Understanding the specific histopathological alterations and cellular mechanisms involved can guide the development of potential therapeutic interventions or preventive measures to mitigate or reduce the adverse effects on liver development caused by CCl_4 exposure. The results of this study may have extensive ramifications for environmental safety, public health, and the creation of tactics to lessen the detrimental effects of CCl_4 and other toxic compounds on liver development.

1.5 JUSTIFICATION OF THE STUDY

The liver is a vital organ involved in numerous physiological functions such as metabolism, detoxification, and synthesis of essential proteins. Investigating how CCl_4 exposure affects the developing liver in Wistar rat dams provides crucial insights into the organ's growth, cellular structure, and potential disruptions caused by this toxic compound. Understanding these effects on liver histology during the critical developmental stages is fundamental to comprehend the potential long-term impacts on overall health. CCl_4 is well-documented as a teratogen, meaning it can cause birth defects. Previous research has linked CCl_4 exposure to adverse effects on fetal development. However, the specific impact of CCl_4 on the histology and structural development of the liver in Wistar rat offspring remains inadequately explored. Investigating the histological changes caused by CCl_4 exposure can reveal potential structural alterations, cell damage, and developmental abnormalities in the liver.

The liver plays a crucial role in maintaining homeostasis and overall health. By examining the effects of CCl4 on liver histology, it may provide clues to how CCl4-

induced liver damage contributes to pregnancy termination, informing the development of preventive strategies. Given the liver's significance in overall health, identifying and understanding potential histological changes induced by CCl4 in the liver can pave the way for the development of preventive measures. If CCl4 exposure is found to negatively impact liver histology, this research can guide the formulation of interventions or treatments to mitigate these effects, potentially reducing the incidence of CCl_4-induced abortions and birth defects.

CHAPTER TWO

LITERATURE REVIEW

2.1 CHEMICAL OF THE STUDY: CCl_4

2.1.1 Description and Properties of CCl_4

Carbon tetrachloride (CCl_4) is a manufactured chemical that does not occur naturally in the environment (Klaassen and Liu, 1998). CCl_4 is a clear (colorless), heavy liquid that evaporates readily, with a distinct sweet characteristic odor similar to chloroform. CCl_4 is classified as a volatile organic compound (VOC) (Bahmani *et al.,* 2021).

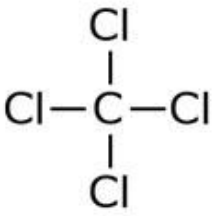


Fig. 2.1: *Carbon tetrachloride chemical structure* (Bahmani *et al.,* 2021)

CCl_4 is composed of one carbon atom bonded to four chlorine atoms, forming a tetrahedral structure (Bahmani *et al.,* 2021). It has a molecular formula of CCl_4 and a molar mass of 153.82 g/mol. The compound is non-flammable, but it can support combustion under certain conditions (Penny *et al.,* 2010). CCl_4 is highly volatile, evaporating readily at room temperature, and is insoluble in water but soluble in organic solvents. It is fairly stable in the presence of air and light. Decomposition of carbon tetrachloride forms phosgene, carbon dioxide, and hydrochloric acid (Rebey *et al.,* 1998; Safer, 2017). It reacts explosively with aluminum powder and some other reactive metals, and, in the presence of peroxides or light, with unsaturated compounds (Rebey *et al.,* 1998). It has been widely used in various industrial

applications, including as a solvent, refrigerant, foam-blowing agent, fire extinguishing agent, and as a precursor in the production of chlorofluorocarbons (CFCs). It is also used in the manufacture of paints, ink, plastics, semi-conductors and petrol additives, as a solvent in and as a grain fumigant, pesticide, pharmaceutical products and flame retardant (Simmonds *et al.,* 1983; Liu *et al.,* 1993). As a result of its widespread use, CCl_4 is a common contaminant of ground and surface waters and air where it persists for years. Therefore, CCl_4 is now of greatest concern as an environmental contaminant (Sanzgiri, 1997). However, due to its high toxicity and potential for environmental and health hazards, the use of CCl_4 has been significantly restricted or banned in many countries (Racicot *et al.,* 1975; Penny *et al.,* 2010). It is listed as a hazardous substance by various regulatory agencies and strict guidelines and safety measures are in place for the handling and disposal of CCl_4 (Penny *et al.,* 2010; Bahmani *et al.,* 2021).

2.1.2 Exposure Routes of CCl_4

Inhalation is the primary means of exposure to CCl_4 in humans (De Bleserc *et al.,* 1995). Human exposure to CCl_4 can occur through inhalation, ingestion, or dermal contact (Marquardt *et al.,* 2012). Occupational exposure may occur in industries that historically used CCl_4, such as dry cleaning, metal degreasing, and chemical manufacturing. Environmental exposure can also occur through contaminated air, water, or soil (Lee *et al.,* 2000).

- **Inhalation**

Breathing in CCl_4 vapors or aerosols is a common route of exposure. This can happen in industrial or occupational settings where CCl_4 is used or produced, or in situations

where CCl_4-containing products are mishandled or released into the environment (De Bleserc *et al.,* 1995). Inhalation of CCl_4 vapors allows the chemical to enter the respiratory system and be absorbed into the bloodstream. When CCl_4 vapor is inhaled, it can enter the lungs and be rapidly absorbed into the bloodstream. From there, it can distribute throughout the body, including to organs such as the liver, kidneys, and brain (Lee *et al.,* 2000; Marquardt *et al.,* 2012).

- **Skin contact**

Direct contact with CCl_4 liquid or its vapors can lead to absorption through the skin. This typically occurs when there is spillage or contact with contaminated surfaces or equipment (Rivera *et al.,* 2001). CCl_4 can penetrate the skin and enter the bloodstream, although the rate of absorption is generally slower compared to inhalation. The absorption rate depends on factors such as the duration of contact, the surface area of exposure, and the condition of the skin (integrity, moisture content, etc.) (Davis and Mehendale, 1980).

- **Ingestion**

Accidental ingestion of CCl_4 can occur if it is present in contaminated food, water, or beverages, although it is relatively rare compared to inhalation and skin contact (Capurro, 1973; Davis and Mehendale, 1980). If CCl_4 is ingested, it can be absorbed from the gastrointestinal tract into the bloodstream. However, due to its low volatility, ingestion is not as significant a route of exposure as inhalation. It can still pose a risk, especially in industrial settings where CCl_4 may be improperly stored or handled (Capurro, 1973; De Bleserc *et al.,* 1995; Marquardt *et al.,* 2012).

2.1.3 Toxicokinetic of CCl_4

- **Metabolism of CCl_4**

The metabolism of carbon tetrachloride (CCl_4) primarily occurs in the liver through a process called biotransformation. The metabolism of CCl_4 involves a series of enzymatic reactions that convert it into various metabolites (Song and Carraway, 2006). The initial step in the metabolism of CCl_4 is its conversion to the highly reactive free radical, trichloromethyl radical (CCl_3), by the action of hepatic cytochrome P450 enzymes, particularly CYP2E1 (Taylor *et al.,* 1991). This reaction requires the presence of oxygen. Once formed, the trichloromethyl radical can react with cellular components such as lipids and proteins, leading to oxidative damage (Capurro, 1973; Song and Carraway, 2006). Peroxidases, such as catalase, can also contribute to the formation of -CCl_3. The trichloromethyl radical (-CCl_3) can react with oxygen to form the trichloromethyl peroxy radical (-$OOCCl_3$) (Zhu *et al.,* 1999). The trichloromethyl peroxy radical (-$OOCCl_3$) can undergo decomposition to produce chlorine (Cl^-) radicals and phosgene ($COCl_2$). Phosgene ($COCl_2$) is a toxic gas that can further react with cellular components and cause damage (Stoyanovsky and Cederbaum, 1996). Chlorine radicals (Cl^-) formed during the decomposition of the trichloromethyl peroxy radical can also react with cellular components, contributing to oxidative stress and tissue damage. In addition to the above reactions, CCl_4 can undergo reductive dechlorination, which is a minor pathway (Taylor *et al.,* 1991; Bahashwan *et al.,* 2015). In this pathway, CCl_4 is converted to chloroform ($CHCl_3$) and other chlorinated compounds. It is important to note that the metabolism of CCl_4 can generate reactive oxygen species (ROS) and free radicals, which can cause

oxidative stress and damage to various organs, particularly the liver (Thrall *et al.,* 2000). The toxic effects of CCl_4 are mainly attributed to its metabolites rather than the parent compound itself (Bahashwan *et al.,* 2015).

- **Elimination of CCl_4**

The elimination of carbon tetrachloride (CCl_4) from the body occurs through various routes. The primary route of elimination is through the lungs via exhalation (Page and Carlson, 1994). CCl_4 is a volatile compound, and once it enters the bloodstream, it can readily evaporate from the lungs during respiration. Other routes of elimination include urine (Thrall *et al.,* 2000), feces (Taylor *et al.,* 1991), and sweat (Song and Carraway, 2006). A small portion of CCl_4 and its metabolites can be excreted in urine (Reyes-Gordillo *et al.,* 2007). This occurs through the filtration of CCl_4 and its metabolites by the kidneys, followed by their elimination in the urine (Thrall *et al.,* 2000). CCl_4 can be eliminated in the feces through a process called biliary excretion (Song and Carraway, 2006). After metabolism in the liver, some CCl_4 metabolites can be transported into the bile, which eventually reaches the intestines. From there, they can be eliminated in the feces (Bahashwan *et al.,* 2015). CCl_4 and its metabolites can be excreted in sweat, although the contribution of this route to overall elimination is relatively minor compared to the lungs, urine, and feces (Page and Carlson, 1994). It is important to note that the elimination of CCl_4 from the body is relatively slow, with a half-life ranging from several weeks to months (Sanzgiri *et al.,* 1997). This slow elimination rate is due to the formation of reactive metabolites and the complex metabolic pathways involved in its breakdown. The prolonged half-life also

contributes to the potential accumulation of CCl_4 in the body, increasing the risk of toxicity and adverse effects (Zhu *et al.,* 1999; Thrall *et al.,* 2000).

Carbon tetrachloride (CCl_4) is a highly toxic chemical that can have adverse effects on various systems in the body (Zhu *et al.,* 1999). CCl_4 is primarily known for its hepatotoxicity. When CCl_4 is metabolized in the liver, it forms highly reactive free radicals that can cause oxidative stress and damage hepatocytes (Itoh *et al.,* 2010). This damage can lead to inflammation, hepatocellular necrosis, and liver fibrosis. Prolonged exposure to CCl_4 can result in liver cirrhosis and even liver cancer (Karakus *et al.,* 2011). Inhalation of CCl_4 vapor can lead to respiratory system toxicity (Das *et al.,* 2014). CCl_4 can irritate the respiratory tract, causing symptoms such as coughing, shortness of breath, and chest pain (Younis *et al.,* 2023). Prolonged exposure can also result in lung damage, including fibrosis and pulmonary edema (Das *et al.,* 2014; Younis *et al.,* 2023). CCl_4 and its metabolites can be filtered by the kidneys and can cause nephrotoxicity (Wiesner *et al.,* 2010). It can lead to kidney damage, impaired kidney function, and the formation of kidney stones. The mechanism of CCl_4-induced kidney toxicity involves oxidative stress, inflammation, and direct damage to renal cells (Ahmed *et al.,* 2015).

Central nervous system (CNS) depression is the immediate result of acute CCl_4 exposure via any route (Palanivel *et al.,* 2008). CCl_4 exposure can have neurotoxic effects. It can cross the blood-brain barrier and cause damage to brain cells (Rivera *et al.,* 2001). Neurological symptoms associated with CCl_4 exposure include headaches, dizziness, confusion, tremors, and in severe cases, seizures and coma (Rom and Markowitz, 2007). CCl_4 exposure can also lead to cardiovascular toxicity (Das *et al.,*

2014). It can cause damage to the heart muscle, resulting in cardiomyopathy and impaired cardiac function (Das *et al.,* 2014; Rangel-Santiago *et al.,* 2016). CCl_4-induced oxidative stress and inflammation can also contribute to the development of cardiovascular diseases (Rangel-Santiago *et al.,* 2016). CCl_4 exposure has been shown to have reproductive toxicity (Karakus *et al.,* 2011; Rangel-Santiago *et al.,* 2016). It can affect fertility and cause adverse effects on reproductive organs (Itoh *et al.,* 2010; Karakus *et al.,* 2011). In animal studies, CCl_4 has been shown to cause testicular toxicity, hormonal imbalance, and reproductive organ damage (Wiesner *et al.,* 2010; Ahmed *et al.,* 2015). Ingestion of CCl_4 can cause gastrointestinal toxicity (Itoh *et al.,* 2010; Rangel-Santiago *et al.,* 2016). It can lead to abdominal pain, nausea, vomiting, and diarrhea (Rangel-Santiago *et al.,* 2016). Direct contact of CCl_4 with the gastrointestinal mucosa can result in tissue damage and ulceration (Zhu *et al.,* 1999). It is important to note that the toxic effects of CCl_4 depend on various factors, including the dose, duration of exposure, route of exposure, and individual susceptibility (Palanivel *et al.,* 2008). Immediate medical attention should be sought in case of CCl_4 exposure or suspected poisoning to minimize the toxic effects and provide appropriate treatment.

2.2 ORGAN OF STUDY: LIVER

The liver plays a crucial role in maintaining homeostasis and performing various metabolic functions in mammals. The anatomical features of the liver, including its lobular structure and vascular supply, are essential for its proper function.

2.2.1 Gross Anatomy

The rat liver has generally 2 surfaces: diaphragmatic and visceral. Despite having multiple lobes, the rat liver has surfaces that are roughly equivalent to lobes that are flat against one another (Kogure *et al.,* 1999). The diaphragmatic convex surface (*facies diaphragmatica*) is in contact with the diaphragm and right abdominal wall (Dulak and Temin, 1973). This surface is covered by the peritoneum. The part of the convex surface that is without peritoneum is referred to as the bare area of the liver (*area nuda*) (Kogure *et al.,* 1999). This surface includes part of the left lateral and medial lobes of the liver (Kogure *et al.,* 1999; Martins and Neuhaus, 2007; Kan and Madoff, 2008). The visceral concave surface (*facies visceralis*) is very rugged, because it is in relation to the guts (stomach, descending duodenum, right colic flexure, jejunum, spleen, pancreas, right kidney, and suprarenal gland) (Kogure *et al.,* 1999). These structures indent the liver and produce impressions of the stomach (*impressio gastrica*), duodenum (*impressio duodenalis*), colon (*impressio colica*), and kidney (*impressio renalis*) (Martins and Neuhaus, 2007). The whole visceral surface is embedded into the peritoneum. The porta of the liver (*porta hepatis*) is located on the visceral surface (Almazroo *et al.,* 2017). The *porta hepatis* goes through the portal vein, hepatic artery and nerves, lymphatic vessels, and common hepatic duct (Kogure *et al.,* 1999; Kan and Madoff, 2008; Zhang *et al.,* 2012; Almazroo *et al.,* 2017). A very well-defined border divides the convex from the concave surface (Kan and Madoff, 2008). There are 4 such borders in the rat liver: right, left, ventral, and dorsal. The left, right, and ventral borders are very sharp but the dorsal border is oblique (Abdel-Misih and Bloomston, 2010). The left border (*margo sinister*) is between the

convex and concave surface on the side of the left lateral and medial lobes of the liver (Kan and Madoff, 2008; McCuskey, 2012). The right border (*margo dexter*) is between the concave and convex surfaces on the right side of the liver, where the right lateral and medial lobes of the liver were situated (Kan and Madoff, 2008). The ventral border (*margo ventralis*) is between the concave and convex surface and is formed by the left and right medial lobes and quadrate lobes of the liver (Kan and Madoff, 2008). The dorsal border (*margo dorsalis*) consists of part of the left and right lateral lobes and caudate lobes. Situated on the dorsal border of the rat liver are the esophageal impression (*impressio esophagea*) and the caudal vena cava (*v. cava caudalis*), located completely inside the liver tissue (Bismuth, 1982; McCuskey, 2012).

2.2.2 Lobes and Notches

The rat liver has six lobes: the caudate, quadrate, left medial (smaller), left lateral (larger), right medial (smaller), and right lateral (bigger), respectively (*lobus hepatis sinister medialis*, *lateralis,* and *lobus hepatis dexter medialis, lateralis,* and *lobus caudatus* et *quadratus*). The caudate lobe is located between the left branch of the portal vein and the caudal vena cava (Kogure *et al.,* 2000; Kumon, 2017). *Processus papillaris* and *processus caudatus*, respectively, are the two processes that make up the caudate lobe. The smaller, more leftward-projecting papillary process (*processus papillaris*), which rises from the porta, is the liver's left side. Preventricular (*pars preventricularis*) and retroventricular (*pars retroventricularis*) are the two distinct phases of this process in rats (Murakami and Hata, 2002). At the renal impression (*impressio renalis*), the massive caudate process spreads to the right side, covering most of the visceral surface of the right lobe of the liver (Murakami and Hata, 2002).

Only interstitial tissue and arteries connect the liver's lateral, larger lobes to the other components. The lateral portion of the liver is located more cranially than the medial, smaller lobes on the right and left sides (Kogure *et al.,* 2000). Between the lateral and medial portions of the left and right lobes, as well as between the right, medial, left, medial, and quadrate lobes, are deep interlobar notches (*incisurae interlobares*). The round ligament (*lig. teres hepatis*) is contained in the deep notch for the round ligament (*incisura ligamenti teretis*), which is located between the left medial lobe and quadrate lobe (McCuskey, 2012; Kumon, 2017).

2.2.3 Ligaments

Rat hepatic ligaments are incredibly fragile. The right abdominal muscles' caudal surface and the convex surface of the diaphragm are connected by a narrow peritoneal fold called the falciform ligament of the liver (*lig. falciforme*) (Doniach and Weinbren, 1952; Madrahimov *et al.,* 2006). The coronary ligament, which inserts around the caudal vena cava exit, is where this ligament runs from the peritoneum on the convex aspect of the liver (Martins *et al.,* 2008). Right and left coronary ligaments (*lig. coronarium dextrum* et *sinistrum*) make up the liver's coronary ligament. The right lobe is connected to the dorsal abdominal wall via the right triangle ligament (Doniach and Weinbren, 1952; Martins *et al.,* 2008; Kumon, 2017). The caudolateral angle of the left lobe is connected to the same area of the abdominal wall via the left triangular ligament. The caudal free edge of the falciform ligament is only slightly thickened by the round ligament (*lig. teres hepatis*) (Kumon, 2017). The gastrosplenic ligament, also known as the greater omentum (*omentum majus*), extends from the greater curvature of the stomach (Doniach and Weinbren, 1952). The hepatogastric and

hepatoduodenal ligaments (*lig. hepatogastricum et hepatoduodenale*) go from the area of the porta to the lesser curvature of the stomach and the proximal part of the duodenum. They are a part of the smaller omentum (*omentum minus*) (Martins *et al.,* 2008; Kumon, 2017).

2.2.4 The Biliary System

Wistar rats have no gallbladder or common bile duct (*ductus choledocus*); they only have a common hepatic duct (*ductus hepaticus communis*), which is formed by right and left hepatic duct (*ductus hepaticus dexter et sinister*) in the portal area, superficially to the portal and arterial branches, because this is an extrahepatic biliary system (Kogure *et al.,* 2000; Murakami and Hata, 2002). Each lobe of the liver has its own biliary ducts, presenting as intrahepatic biliary tracts. The right hepatic duct is formed by the confluence of the duct from the caudate and right lobe (McCuskey, 2012). The left hepatic duct drains the bile from the left and quadrate lobe of the liver. The caudate process drains bile into the duct of the right lateral lobe and the caudate lobe, or directly into the main biliary tract (Almazroo *et al.,* 2017). All hepatic ducts are fused and form a common hepatic duct (*ductus hepaticus communis*), which leads to the duodenum (Zhang *et al.,* 2012). The common hepatic duct is situated ventrally and to the right of the portal vein (*v. portae*) (Doniach and Weinbren, 1952; Zhang *et al.,* 2012).

2.2.5 The Vessels

The liver in Wistar rats receives a dual blood supply through the hepatic artery and portal vein (Bismuth, 1982). The hepatic artery supplies oxygenated blood to the liver, while the portal vein delivers nutrient-rich blood from the gastrointestinal tract

(Abdel-Misih and Bloomston, 2010). These vessels traverse through the liver lobules, giving rise to a complex network of sinusoids that facilitate the exchange of substances between blood and hepatocytes (Abdel-Misih and Bloomston, 2010; Zhang *et al.,* 2012). Lymphatic vessels accompany the hepatic arteries and portal veins and converge to form larger lymphatic vessels. These vessels ultimately drain into the celiac lymph nodes (Abdel-Misih and Bloomston, 2010).

2.2.6 Embryology

On embryonic day 10, the rat liver begins its development (Russell and Snyder, 1968). Its cells acquire the morphological appearance of immature rat liver hepatoblasts on embryonic day 11 (Berthoud *et al.,* 1992). The primitive epithelial cells of the foregut form the hepatic bud, which is divided into a smaller caudal part (*pars cystica*) and a larger cephalic part (*pars hepatica*) (Russell and Snyder, 1968; Warner *et al.,* 1984). The caudal part gives rise to the gallbladder and cystic duct and the cephalic part gives rise to parts of the parenchyma of the liver, intrahepatic ducts, and both (right and left) hepatic ducts (Dulak and Temin, 1973). Except for the cystic bud, most of the structures of the rat embryo are present in the human embryo (Lemaigre and Zaret, 2004). On embryonic day 13 the liver is expanded greatly in size and its position is just caudal to the diaphragm (Russell and Snyder, 1968). On the coronal section, many interlobar spaces are found at the same time. These spaces divided the liver into 4 main lobes: median, right, left, and caudate lobes (Dulak and Temin, 1973). The median lobe is divided into the right and left parts. The right lobe is subdivided into the right cranial and caudal lobes (Warner *et al.,* 1984). On embryonic day 15, the venous duct, post-hepatic caudal vena cava, and portal vein appear in the transverse

section and the liver lobes are demarcated in the coronal section (Russell and Snyder, 1968). On embryonic day 19, there is a marked increase in the number and size of hepatocytes in the rat liver tissue (Dulak and Temin, 1973). On embryonic day 21, the liver achieves its mature adult architecture (Berthoud *et al.,* 1992). The sinusoids of the blood are thin irregular structures between the cords of the liver cells. The rat liver lacks a gall bladder and cystic bud in rats (Dulak and Temin, 1973; Berthoud *et al.,* 1992).

2.2.7 Histology

The histology of a rat's liver is similar to that of other mammalian livers, including humans (Afolayan and Yakubu, 2009). The liver is composed of numerous lobules, which are the basic functional units of the organ (Dardouri *et al.,* 2016). Each lobule is roughly hexagonal in shape and consists of hepatocytes (liver cells) arranged in a radial manner around a central vein (Buraimoh *et al.,* 2011). These hepatocytes are the predominant cell type in the liver. They are polygonal in shape and have a granular appearance due to numerous cytoplasmic organelles, such as mitochondria and endoplasmic reticulum (Afolayan and Yakubu, 2009; Amini *et al.,* 2012). Hepatocytes perform various metabolic functions, including protein synthesis, detoxification, and bile production (Iyare *et al.,* 2017). The Sinusoids are specialized capillaries that run between rows of hepatocytes within the lobule (Buraimoh *et al.,* 2011). They are lined by fenestrated endothelial cells and have a discontinuous basal lamina. Sinusoids receive blood from the hepatic portal vein (carrying nutrient-rich blood from the digestive system) and the hepatic artery (supplying oxygenated blood). Also, Kupffer cells are specialized macrophages located within the sinusoids (Dardouri *et al.,* 2016).

They play a crucial role in immune defense by engulfing and eliminating foreign particles, pathogens, and old/damaged red blood cells (Afolayan and Yakubu, 2009). The Bile canaliculi are small channels located between adjacent hepatocytes. They collect bile synthesized by hepatocytes and transport it toward the bile ducts. The Bile ducts are thin tubes that collect bile from the bile canaliculi (Buraimoh *et al.,* 2011; Shahabi *et al.,* 2018). They merge and form larger ducts, ultimately leading to the common bile duct, which transports bile to the duodenum (part of the small intestine). The liver exhibits regional variation in function and structure (Shahabi *et al.,* 2018). The periportal zone, also known as zone 1, is closest to the portal triad (consisting of a branch of the hepatic artery, hepatic portal vein, and bile duct) (Iyare *et al.,* 2017). The perivenous zone, also known as zone 3, is located around the central vein (Afolayan and Yakubu, 2009; Dardouri *et al.,* 2016). These zones show differences in metabolic activity and susceptibility to certain toxins or diseases (Afolayan and Yakubu, 2009; Iyare *et al.,* 2017; Shahabi *et al.,* 2018). It's important to note that there can be variations in the appearance of liver tissues depending on factors such as the age of the rat, its physiological state, and any underlying diseases or experimental conditions (Buraimoh *et al.,* 2011; Iyare *et al.,* 2017).

2.3 REVIEW OF RELATED LITERATURE

CCl_4 is a common environmental pollutant. Workers are at high risk of exposure to high levels through inhalation and dermal contact. On the other hand, the general population may be exposed to low levels of CCl_4 through inhalation from the atmospheric environment (Liu *et al.,* 2009). Because of its harmful effects, its uses are

now banned and it is only used in some industrial applications. mentioned that CCl_4 is also well known for hepatic toxic actions. These free radicals can generate lipid peroxide which may cause cell membrane damage, alteration in enzyme activity, and finally induction of hepatic injury and necrosis. Höhme et al. (2007) reported that CCl_4 initially induced cell death of a pericentral ring of hepatocytes followed by destruction of the characteristic microarchitecture of the hepatic lobules. Uskoković-Marković *et al.* (2007) mentioned that the induced liver necrosis by CCl_4 is an example of a model for experimental liver necrosis and cirrhosis caused by oxygen free radicals. CCl_4-induced hepatic injury is a very classic model widely used for hepatoprotective drug screening. The acute hepatotoxicity of CCl_4 lies in its biotransformation to trichloromethyl free radical (CCl_3) or trichloro peroxyl radical ($CCl_3O_2^-$) produced by the mixed-function cytochrome P450 (CYP) oxygenase system of the endoplasmic reticulum, which causes oxidative stress and membrane damage (Huang *et al.,* 2012). Ijiri *et al.* (2014) concluded that CCl_4 facilitates the generation of hepatotoxins that can result in morphologic abnormalities and these abnormalities are reasonably characteristic and reproducible for each particular toxin. They also stated that TNF-α may participate in CCl_4 -induced liver injury. Li *et al.* (2014) mentioned that CCl_4 is commonly used as a model toxicant to induce chronic and acute liver injuries. Acute liver injury was successfully induced by CCl_4 as revealed by histopathological results and a significant increase in ALT and AST. CCl_4 also caused a decrease in some of the amino acids. Ding *et al.* (2005) induced liver fibrosis by injecting CCl_4 IP in rats for eight weeks. They observed disruption of tissue architecture, large fibrous septa formation, pseudo-lobe separation, and collagen

accumulation. Electron microscopic examination indicated distorted cell organoids. These changes were accompanied by an increase in the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), while albumin decreased significantly. Sahreen *et al.* (2011) and Veidal *et al.* (2011) reported that the liver is a target organ for CCl_4 toxicity due to its detoxifying function in protecting the body. CCl_4 is a well-known hepatotoxic industrial solvent so it is used in diverse experimental models. Liver fibrosis was induced by CCl_4 in adult male albino rats in a study done by Ahmed *et al.* (2011). They mentioned that CCl_4 increased serum liver enzymes (ALT, AST, and alkaline phosphatase, lactate dehydrogenase, level of nitric oxide, tumor necrosis factor-alpha (TNFα), and liver malondialdehyde content, collagen fiber percent and decreased liver reduced glutathione content as an endogenous antioxidant. They added that histopathological changes induced by CCl_4 include regenerative nodules, deteriorated parenchyma, and the lobules infiltrated with fat and structurally altered.

CHAPTER THREE

MATERIALS AND METHOD

3.1 EXPERIMENTAL ANIMALS

Nulliparous Wistar rats purchased and bred at the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, were used for this study. The animals were kept in polypropylene cages at room temperature with 12-hour light and dark cycle photoperiodicity. The animals were left to acclimatize for two weeks before the commencement of the experiment. The animals were fed with Topfeeds Growers Pellets (manufactured by Premier Feed Mills Company Limited, 1 Eagle Flour Road, Lagos/Ibadan Expressway Toll point, Ibadan, Oyo State, Nigeria) and clean tap water *ad libitum*. The animals were weighed weekly before commencement and throughout the duration of the experiment using a digital weighing scale calibrated in grams and recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

Thirty (30) adult female Wistar rats weighing between 160g and 180g were used for this experimental study. A regular estrous cycle was established in all the groups before the commencement of administration for fourteen days i.e. approximately 3

cycles. Estrous cycles of both control and treatment groups were monitored daily by vaginal lavage (Marcondes *et al.,* 2002). Vaginal secretion was collected after douching with 0.2 ml of normal saline (0.9% NaCl) contained in a smooth plastic pipette. A small drop of the cell suspension in the pipette was placed on a clean glass slide and examined under a light microscope without the use of a condenser lens at X10 and X40 magnification. Vaginal smears were then assessed once each day between 9:00 and 10:00 a.m. The proportion of characteristic cell types; leucocytes, cornified and epithelial cells, were used to determine the phases of the oestrous cycle according to Mandl (1951): proestrus was identified by the predominance of epithelial cells which were round and nucleated; oestrous was mainly characterized by cornified cells which were irregular and non-nucleated; met-oestrous by cornified and leucocytic cells and diestrous by main leucocytes which were little round cells. The length of oestrous cycles and the duration of each phase of the cycle were recorded as described by Makonnen et al. (1997). Only animals that presented with a regular four days cycle (i.e. oestrous phase at four days intervals) were selected for this study. Animals with four to five days of oestrous cycle were paired overnight with sexually active males in the ratio of 2:1. Successful mating was confirmed by the presence of a vagina plug and/or sperm in the vagina smear the following morning between 9:00 and 10:00 hours. The day sperm cells were found in the vagina smear was considered Gestational Day (GD) 0. The rats were weighed daily and physically observed.

3.2 EXPERIMENTAL DESIGN

The thirty Wistar rat dams were randomly divided into two groups (A & B) of 15 rats each. Group A served as the control that was administered with 0.2ml of normal saline orally on GD8. Group B animals received 200mg/Kg of CCl4 (Bruckner *et al.,* 1986). All animals were allowed free access to feed and water. Four animals were sampled from each group on GD15, GD17, and GD19. Each rat was laparotomised under chloroform anesthesia. The uterine horns were exteriorized and incised at the greater curvature of the horns. Fetal liver tissues were harvested from each group for histological assessment.

The harvested tissues were preserved in 10% phosphate-buffered formalin for histopathology. The tissues were processed via the paraffin wax embedding method of Drury and Wallington (1980). They were dehydrated for one hour each at room temperature through ascending grades of ethanol; 70% ethanol, 90% ethanol, Absolute ethanol I, and Absolute ethanol II. Dehydrated tissues were cleared at room temperature in two changes of xylene for one hour in each change. The tissues were cleared at room temperature in two changes of xylene for one hour in each change and finally embedded in paraffin wax multi-block plastic embedding Molds. The paraffin-blocked tissues were trimmed and mounted on a wooden chaunk for sectioning on a rotary microtome. Sections of 5µm thickness were produced from the tissue blocks using a rotary microtome (Bright B5143, Huntington, England). The Sections were transferred into the water bath (40^{o}C) to allow the spreading of the folded ribbon of sections. These sections were mounted on new clean glass slides. These were dried at 40^{o}C on a slide drier to enhance adherence of the sections to the slides.

3.3 HAEMATOXYLIN AND EOSIN STAINING PROCEDURE

Tissue sections were deparaffinized in two changes of xylene for two minutes in each change and passed through two changes of absolute alcohol for four minutes each. They were hydrated using a series of descending grades of alcohol until the water was used. Procedures of Haematoxylin and Eosin adopted in the sections were described by Drury and Wallington (1980) and Scheehan and Hrapchak (1980). The sections were:

- Dewaxed in two changes of xylene for two minutes in each change;
- Rehydrated in descending grades of alcohol (absolute II, absolute I, 95%, 90%, 70%, and 50% ethanol) for two minutes each;
- Rinsed in distilled water for three minutes
- Stained in hematoxylin for 15-20 minutes
- Excess hematoxylin stain was removed by rinsing well in running tap water for two to three minutes (sections were examined microscopically at this stage to confirm a sufficient degree of staining);
- Differentiated in acid alcohol (0.5% HCl in 70% ethanol for two to three minutes;
- Rinsed well in running water for 10-15 minutes;
- Counterstained in 1% aqueous eosin for two to four minutes;
- Excess stain was washed off in running water and examined under a microscope;

- Dehydrated rapidly in ascending grades of ethanol (50% through absolute ethanol), cleared in xylene, and mounted in a synthetic resin medium (DPX).

3.4 STATISTICAL ANALYSIS

Data were analyzed using IBM statistical package for social sciences. Results were presented as mean ± standard error of the mean (mean ± SEM). The parameters for all the groups were compared using students' t-test (two-tailed, assuming equal variance). Differences in mean were considered statistically significant at a 95% confidence level (that is when probability would be less than 0.05 {$P<0.05$}).

CHAPTER FOUR

RESULTS

4.1 HISTOLOGICAL RESULTS

The pregnant Wistar rats in groups A and B were used for this assessment. The findings were demonstrated in the plates below.

Plate 4.1: Photomicrograph of a section of the liver of control group GD15 showing developing hepatoblasts (*) and canalized central vein (CV) H&E

Plate 4.2: Photomicrograph of a section of liver of ccl4 treated group on GD15 showing persistent erythroblastic island (EI) H&E

Plate 4.3: Photomicrograph of a section of the liver of control group GD17 showing patent central vein (CV) H&E

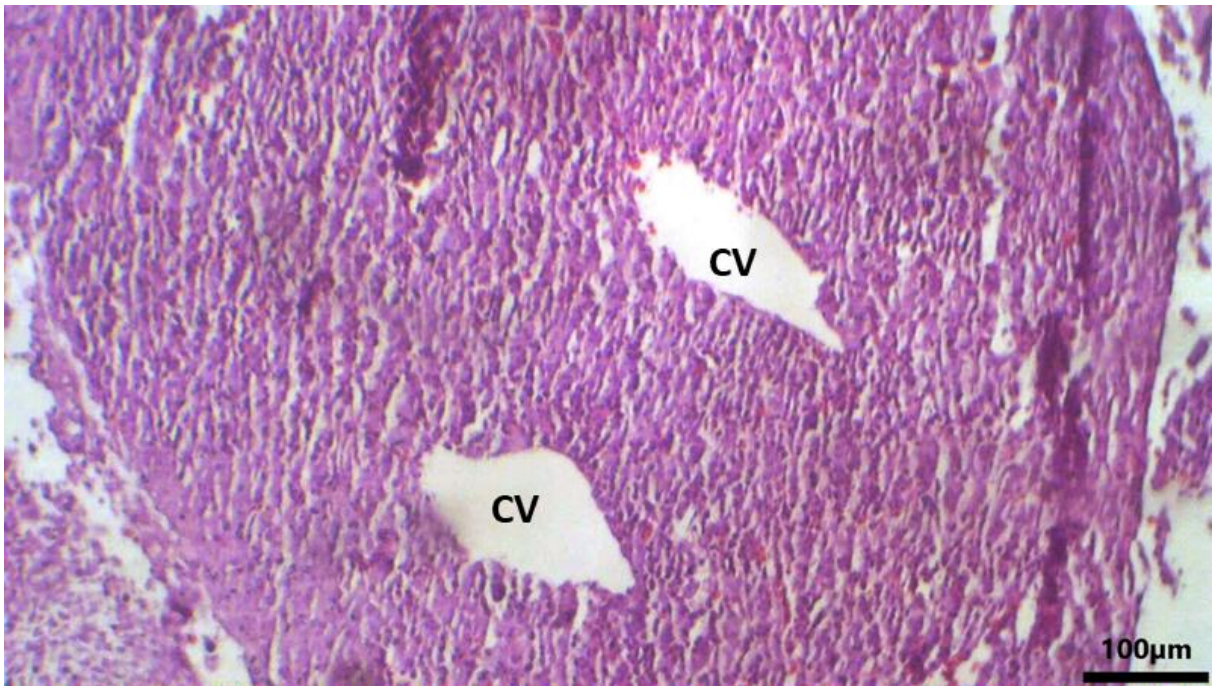
Plate 4.4: Photomicrograph of a section of liver of ccl4 treated group on GD17 showing occluded central veins (encircled) H&E

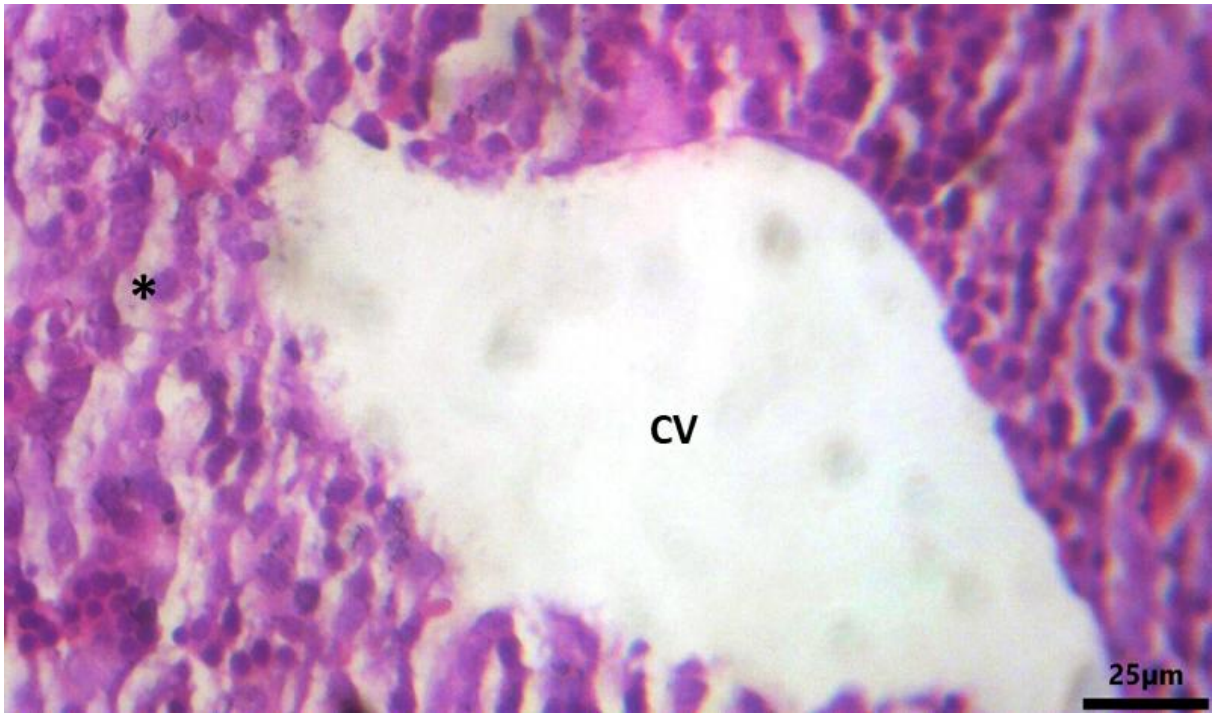
Plate 4.5: Photomicrograph of a section of the liver of control group GD19 showing patent central vein (CV) H&E

Plate 4.6: Photomicrograph of a section of liver of ccl4 treated group on GD19 showing congested vessel (CV)H&E

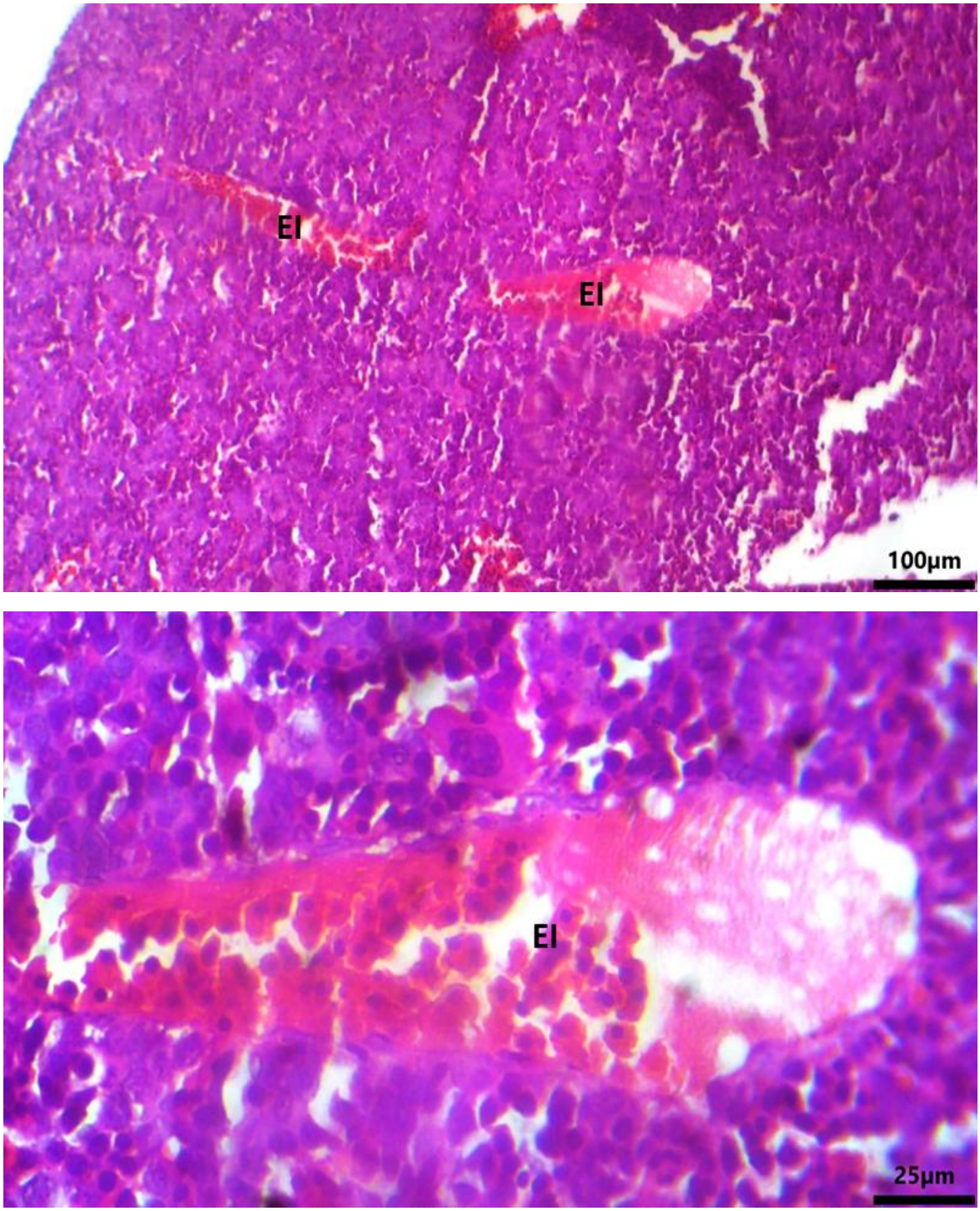
Plate 4.7: Photomicrograph of a section of the liver of control group GD21 showing canalized central vein (CV) H&E

Plate 4.8: Photomicrograph of a section of liver of ccl4 treated group on GD21 showing occlusion of central veins (encircled) H&E

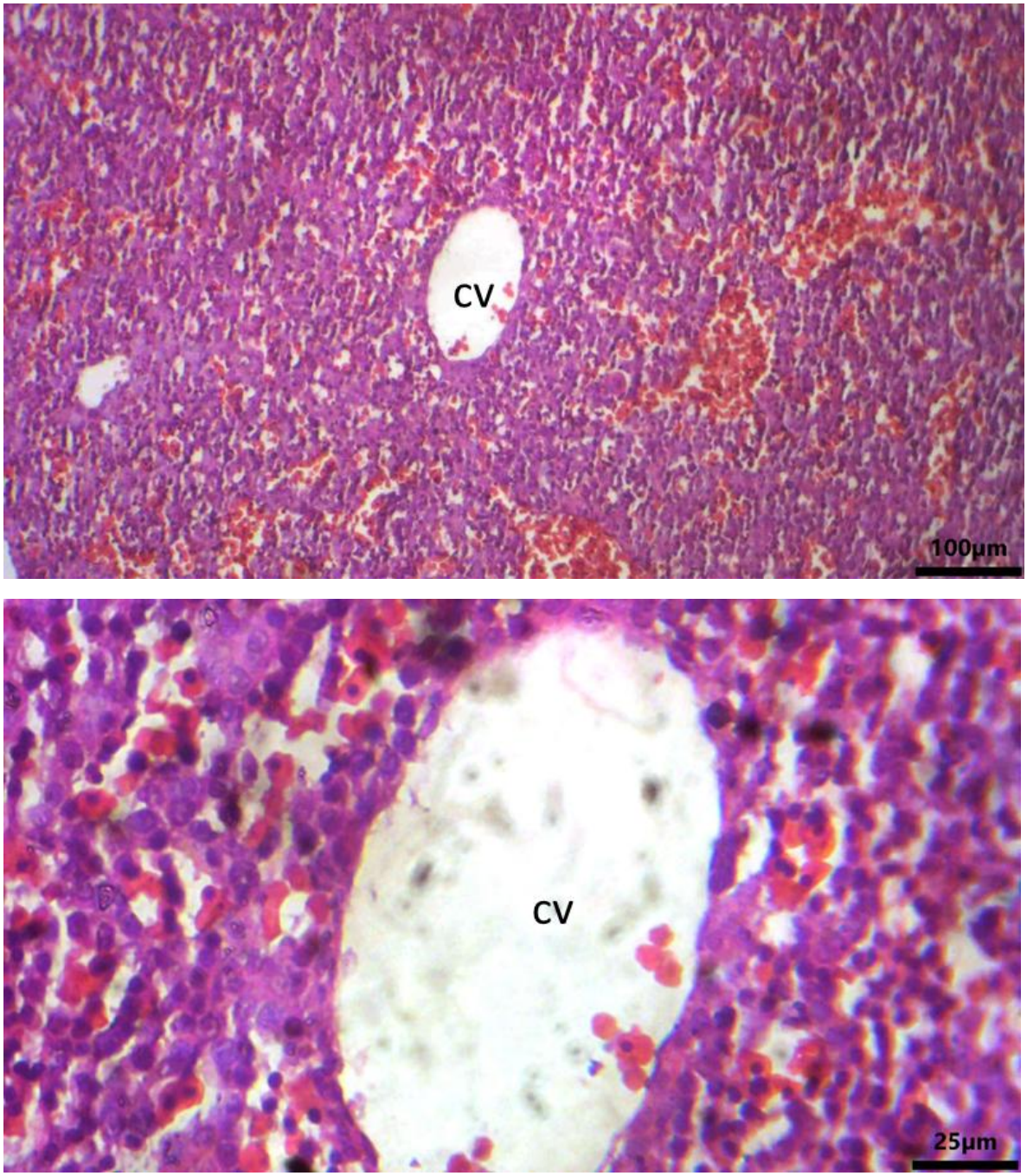




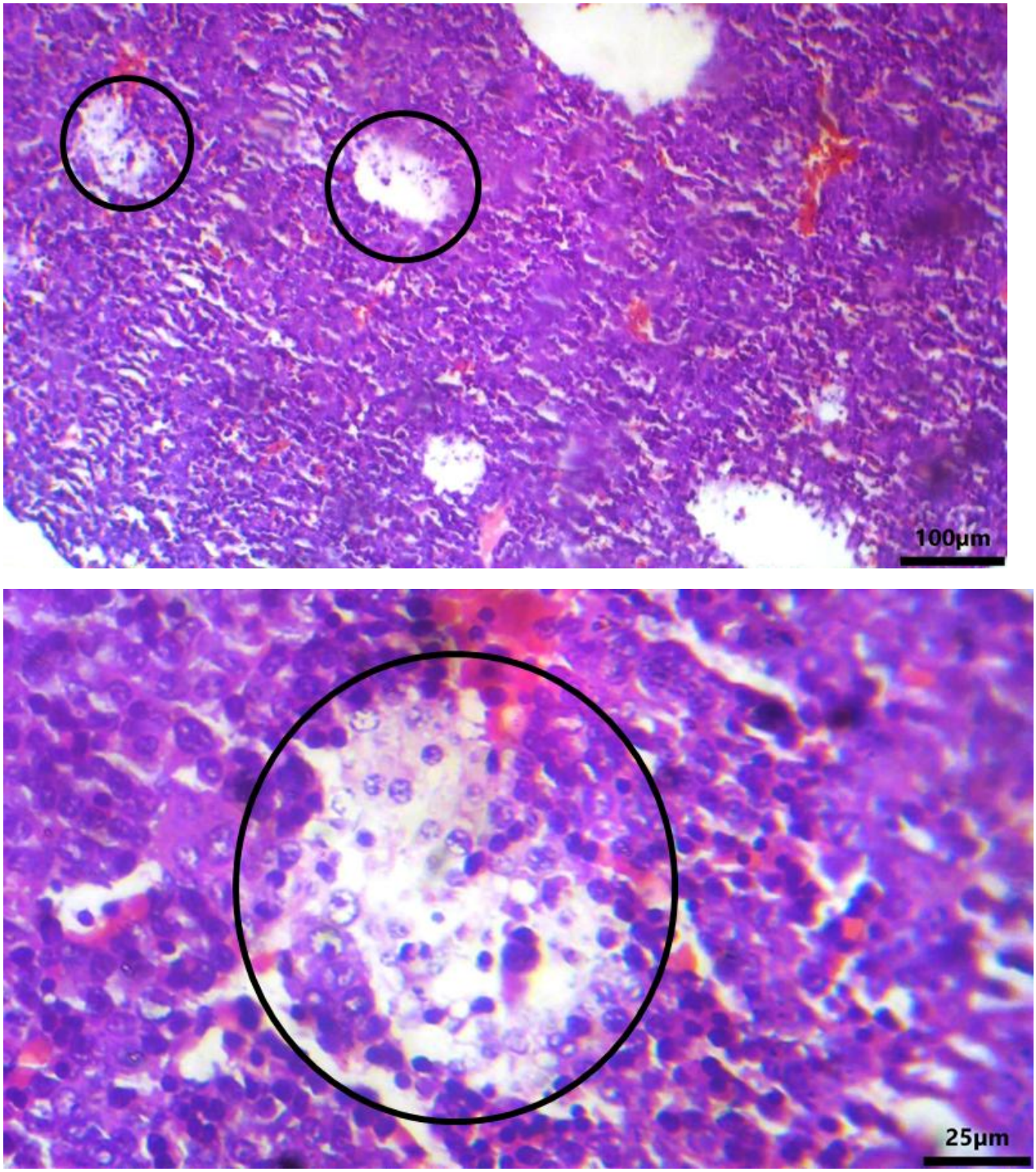
Photomicrograph of a section of the liver of control group GD15 showing developing hepatoblasts (*) and canalized central vein (CV) H&E



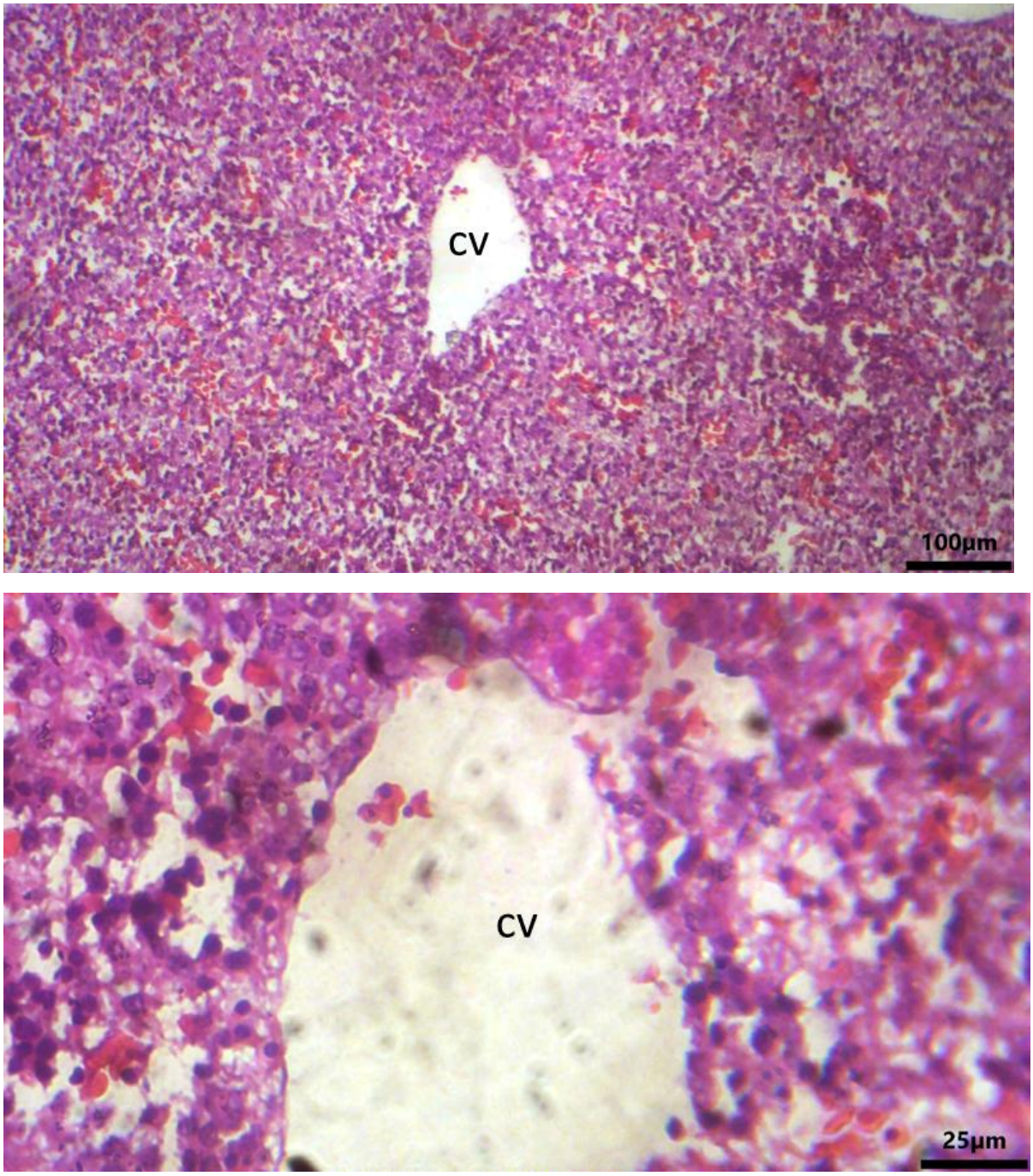
Photomicrograph of a section of liver of ccl4 treated group on GD15 showing persistent erythroblastic island (EI) H&E



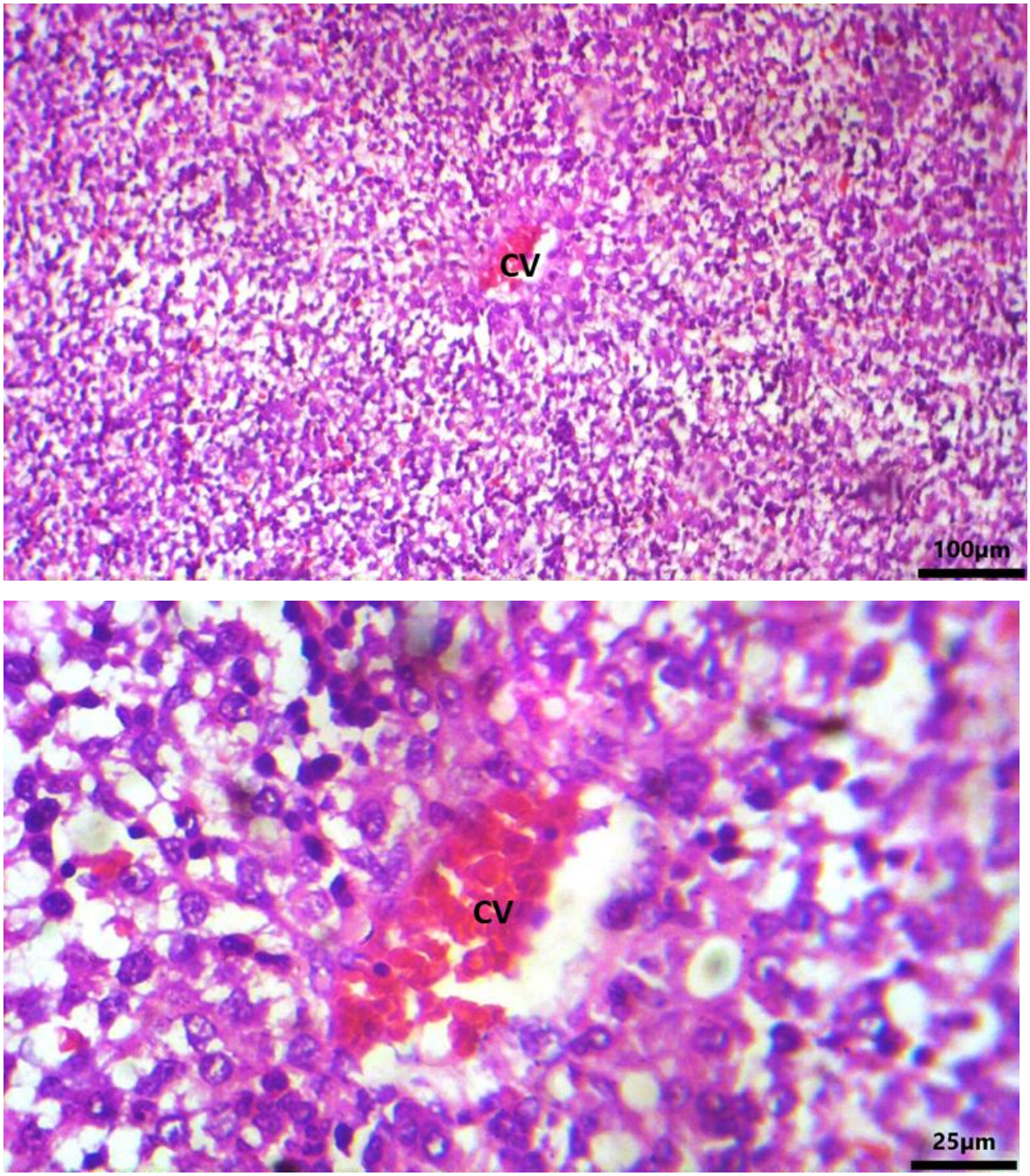
Photomicrograph of a section of the liver of control group GD17 showing patent central vein (CV) H&E



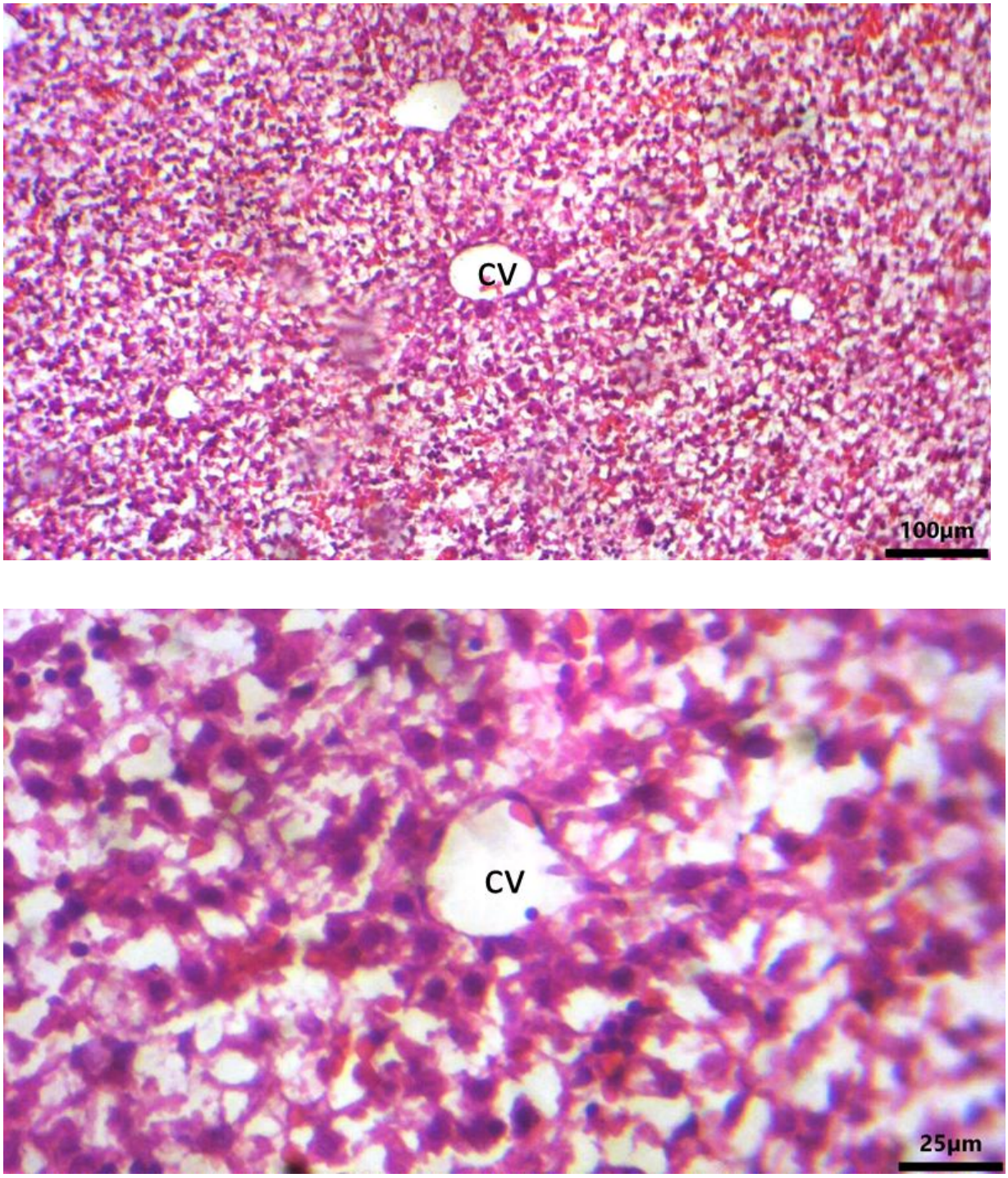
Photomicrograph of a section of liver of ccl4 treated group on GD17 showing occluded central veins (encircled) H&E



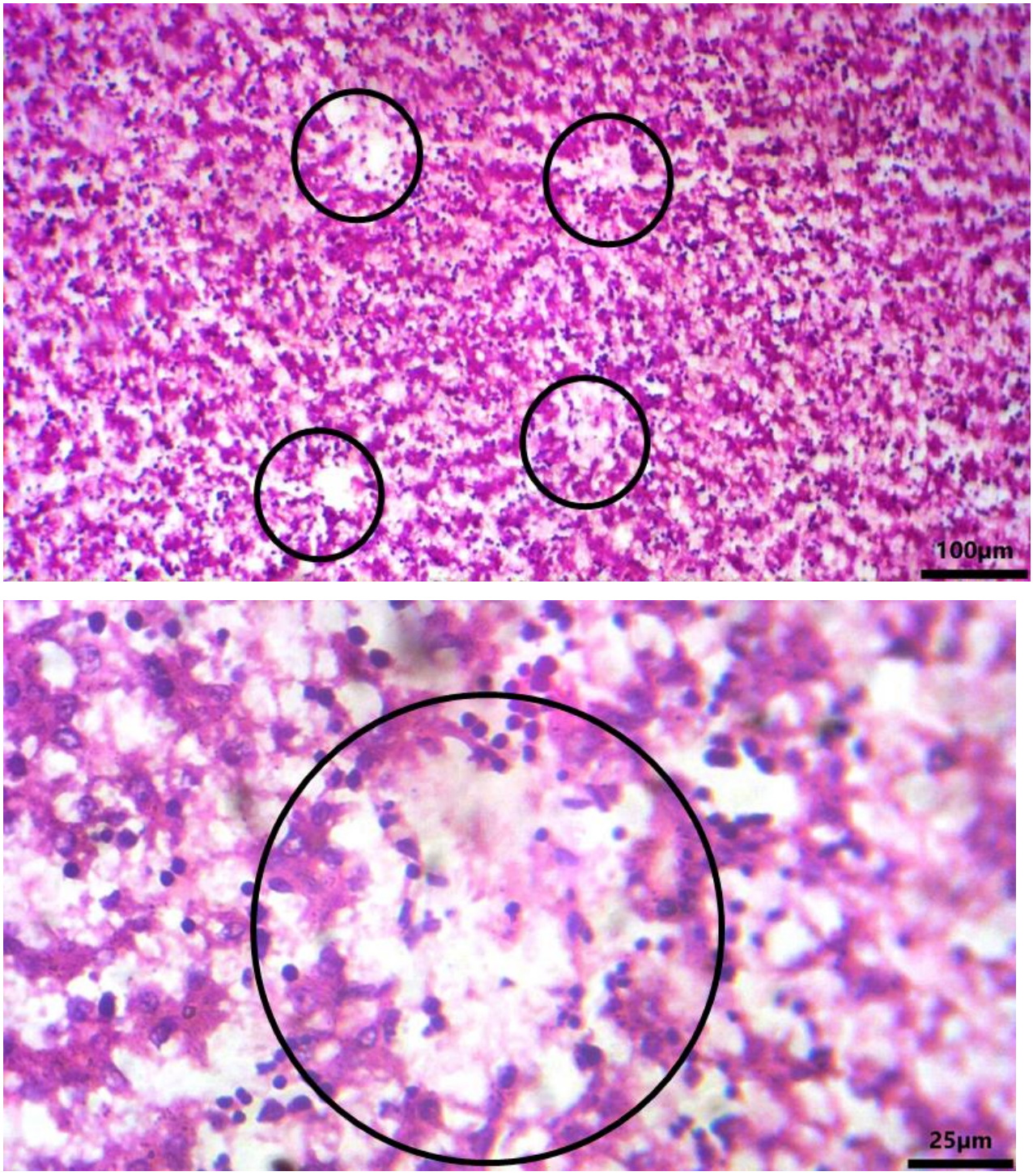
Photomicrograph of a section of liver of control group GD19 showing patent central vein (CV) H&E



Photomicrograph of a section of liver of ccl4 treated group on GD19 showing congested vessel (CV)H&E



Photomicrograph of a section of the liver of control group GD21 showing canalized central vein (CV) H&E



Photomicrograph of a section of liver of ccl4 treated group on GD21 showing occlusion of central veins (encircled) H&E

CHAPTER FIVE

DISCUSSION CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The fetal liver plays a vital role in the hematopoietic process and supports the development of hepatoblasts and vascular structures.

On GD15, the control group displayed developing hepatoblasts and a canalized central vein. This observation is consistent with normal fetal liver development, where hepatoblasts play a pivotal role in forming the liver's parenchyma, and a patent central vein indicates a healthy vascular system. In contrast, the CCl_4-treated group, exhibited persistent erythroblastic islands (EI). The presence of these islands implies an interruption in the normal hepatic maturation process. This disruption can have a profound impact on both hematopoiesis and liver development.

On GD17, the control group presented a patent central vein. This finding is indicative of a healthy vascular structure and is in line with the expected progression of fetal liver development. However, in the CCl_4-treated group, the liver section revealed occluded central veins (encircled). This suggests that CCl4 exposure interferes with normal vascular development, leading to potential issues with blood flow and tissue oxygenation within the fetal liver.

The control group on GD19, showed a patent central vein (CV). This observation indicates a healthy and well-developed vascular structure in the fetal liver. In the CCl_4-treated group, the liver section depicted a congested vessel. The congestion of the vessel is a clear sign of disrupted blood flow within the liver, suggesting that CCl_4 exposure impacts the vascular integrity and function of the fetal liver.

On GD21, the control group displayed a liver section with a canalized central vein. This is in line with the normal maturation process of the fetal liver and its vascular system. In contrast, the CCl_4 -treated group presented occlusion of central veins. This indicates a persistent disruption in the vascular development of the fetal liver under the influence of CCl_4.

The results of this histological assessment in the context of CCl_4 exposure on fetal liver development align with previous studies that have explored the impact of hepatotoxic agents on hepatic and vascular structures during gestation (Amini *et al.,* 2012; Bahashwan *et al.,* 2015). From the findings of this study, CCl_4 exposure led to alterations in vascular structures, with occluded central veins and congested vessels observed. These findings emphasize the vulnerability of fetal liver development to hepatotoxic agents, which can disrupt the natural progression of hepatic and vascular maturation. Understanding the mechanisms by which CCl4 interferes with fetal liver development is essential to better manage and mitigate the risks associated with such exposures during pregnancy. The disruption of hepatoblast development and vascular integrity in the fetal liver can have profound implications for both hematopoiesis and overall fetal health.

5.2 CONCLUSION AND RECOMMENDATION

This study showed that carbon tetrachloride (CCl_4) has teratogenic potential against liver development in Wistar rats. It is recommended that further studies be carried out on postnatal development of the liver following intrauterine exposure to CCl_4. Future research is warranted to delve deeper into the mechanisms and potential preventive measures to protect fetal liver development from hepatotoxic insults.

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