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Hepato-renal Protective Effect of Antioxidants (Curcuma and Black Pepper) Against CCL_4 Induced Toxicity in Male Albino Rats

Thesis submitted to the University of Zawia for the degree of Master of Sciences in Zoology

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بسم الله الرحمن الرحيم

﴿ قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العظيم

سورة البقرة / الآية "32"

Abstract

The liver is the main target for CCl_4 toxicity while the kidney is the main site of CCl_4 accumulation. Repeated administration of CCl_4 to the experimental animals not only produces liver cirrhosis but also pathological changes in different organs such as lung, spleen, and kidneys. Medicinal plants and herbs play an important role in the prevention and treatment of liver and kidney diseases._Curcumin and black pepper are widely used as a spice and in Ayurvedic medicine.

The present study aimed to investigate the protective effect of curcumin and black pepper extract against hepatorenal toxicity induced by CCl_4 in male albino rats.

24 male adult Fischer rats were used in the current study. The animals were divided into 4 groups randomly (Each group of 6 rats). Group I: (The control group), animals were supplied with drinking water from the tap and a natural diet for 28 days. Group II: (The carbon tetrachloride group), rats were injected intraperitoneally with 1.5 mg/kg/day of diluted carbon tetrachloride (3 days a week) for 28 days. Group III: Rats in this group were treated with curcumin and black pepper extract by a daily dosage for 28 days. Group IV: This group was treated with carbon tetrachloride intraperitoneally 3 days a week with daily curcumin and black pepper extract. At the end of the experiment and 24 hours after the last dose, all animals were anesthetized with ether, and blood samples were collected by heart puncture. The blood samples were collected in a clean dry tube and centrifuged at 3000 rpm for 10 minutes then, the serum was separated and biochemical measurements were carried out. Serum ALT, AST, and ALP activities, the concentrations of urea, creatinine, Na^+ and K^+ were measured. The small

pieces of the liver and kidney were fixed in formalin (10%) for histological examination. Statistical significance was tested by the Unpaired Student's t-test. Dunnett's Multiple Comparison Test with a one ways analysis of variance (ANOVA) was used for multiple comparisons. This was done with GraphPad Prism 7.0 software.

Intraperitoneal injection of CCl_4 to male albino rats caused a significant ($P<0.01$) increase in serum activities of ALT, AST, ALP, and concentrations of serum urea, creatinine, and K^+, and a significant ($P<0.01$) decrease in Na^+ concentrations when compared with the control group. The liver sections showed a dilatation, congestion, damage in the lining endothelium, and fibrosis in the wall of the central vein, disorganized, and focal necrosis, some hepatocytes appear with vacuoles in cytoplasm, congestion of some blood sinusoids contain activated kupffer cells, a leukocytic infiltration, and inflammatory cells around portal blood vessels, and thick the wall of hepatic artery and bile ducts. Also, the kidney sections showed a partial destruction of the brush border and desquamated cells and the presence of epithelial debris inside the lumens of the proximal convoluted tubules, and some of which contained red blood cells. The cortex area of the kidney showed shrinkage of some glomeruli with wide capsular space, and congestion of blood vessels and interlobular spaces. Co administration of rats with CCl_4 concurrently with curcumin and black pepper extract resulted in a significant ($P<0.01$) decrease in the activities of serum ALT, AST, ALP and concentrations of serum urea, creatinine, and K^+, and a significant ($P<0.01$) increase in Na^+ when compared with the CCl_4 group. Treatment of rats with curcumin and black pepper extract plus CCl_4 caused improvement in the histological structure of the liver and kidney tissues when compared with the CCl_4-treated groups.

It can be concluded that, CCl_4 has adverse effects on the liver and kidneys. Curcumin and black pepper extract were able to protect the liver and kidneys against these effects. So, individuals exposed to hepatorenal toxic agents should be advised to take this extract.

المستخلص

الكبد هو الهدف الرئيسي لسمية CCl_4 بينما الكلى هي الموقع الرئيسي لتراكمه. إن الإعطاء المتكرر لـ CCl_4 لحيوانات التجارب لا ينتج عنه تليف الكبد فحسب، بل ينتج أيضًا تغيرات مرضية في أعضاء الجسم المختلفة مثل الرئة والطحال والكلى. تلعب النباتات والأعشاب الطبية دورًا مهمًا في الوقاية والعلاج من أمراض الكبد والكلى. يستخدم الكركمين والفلفل الأسود على نطاق واسع كتوابل وفي الطب الهندي القديم.

هدفت الدراسة الحالية إلى معرفة التأثير الوقائي لمستخلص الكركم والفلفل الأسود ضد السمية الكبدية–الكلوية التي يسببها CCl_4 في ذكور الجرذان البيضاء.

تم استخدام 24 من ذكور الجرذان البالغة في الدراسة الحالية. تم تقسيم الحيوانات إلى 4 مجموعات بشكل عشوائي (كل مجموعة من 6 جرذان). المجموعة الأولى: (المجموعة الضابطة)، زودت فيها الحيوانات بمياه الشرب من الصنبور واتباع نظام غذائي طبيعي لمدة 28 يومًا. المجموعة الثانية: (مجموعة رابع كلوريد الكربون)، حقنت الجرذان داخل الصفاق بـ 1.5 مجم / كجم / يوم من رابع كلوريد الكربون المخفف (3 أيام في الأسبوع) لمدة 28 يومًا. المجموعة الثالثة: عولجت الفئران في هذه المجموعة بمستخلص الكركمين والفلفل الأسود بجرعة يومية لمدة 28 يومًا. المجموعة الرابعة: عولجت هذه المجموعة برابع كلوريد الكربون داخل الصفاق 3 أيام في الأسبوع مع مستخلص الكركمين والفلفل الأسود يومياً لمدة 28 يومًا. في نهاية التجربة وبعد 24 ساعة من آخر جرعة، تم تخدير جميع الحيوانات بالإيثير، وتم جمع عينات الدم عن طريق ثقب القلب. جمعت عينات الدم في أنابيب جافة ونظيفة وطردت مركزياً عند 3000 دورة في الدقيقة لمدة 10 دقائق ثم تم فصل المصل لعمل القياسات الكيموحيوية. تم قياس أنشطة الإنزيمات ALT وAST وALP، تم قياس تركيزات اليوريا والكرياتينين و Na^+ و K^+

في مصل الدم. تم تثبيت قطع صغيرة من الكبد والكلى بالفورمالين (10٪) للفحص النسيجي. تم اختبار الدلالة الإحصائية من خلال (T–Student test). ثم استخدام (Dunnett's test) للمقارنة المتعددة مع تحليل التباين أحادي الطرق (ANOVA) لإجراء مقارنات متعددة. تم ذلك باستخدام برنامج GraphPad Prism 7.0.

أدى حقن ذكور الجرذان البيضاء داخل الصفاق بـ CCl_4 إلى حدوث زيادة معنوية ($P<0.01$) في أنشطة الإنزيمات ALT، AST، ALP وتركيزات اليوريا والكرياتينين و K^+، وانخفاض معنوي ($P<0.01$) في تركيز Na^+ في مصل الدم بالمقارنة مع المجموعة الضابطة. أظهرت القطاعات النسيجية للكبد أن اتساع، احتقان، تلف وتليف بطانة الوريد المركزي، ونخر غير منظم، ونخر بؤري، ووجود فجوات سيتوبلازمية في بعض الخلايا الكبدية، واحتقان وخلايا كوبفر نشطة في بعض الجيوب الدموية، ووجود ارتشاح بخلايا الدم البيضاء والخلايا الالتهابية حول الأوعية الدموية البابية، وسماكة جدار الشريان الكبدي والقنوات الصفراوية. كما أظهرت القطاعات النسيجية للكلى تدميرًا جزئيًا في بطانة الأنابيبات الملتفة القريبة ووجود حطام طلائي داخلها وبعضها يحتوي على خلايا الدم الحمراء. أظهرت منطقة قشرة الكلى انكماشًا في بعض الكبيبات مع وجود اتساع في تجويف حفظة بومان، واحتقان في الأوعية الدموية وبين الأنيبيبات البولية. أدى حقن الفئران بـ CCl_4 بالتزامن مع تناول مستخلص الكركمين والفلفل الأسود إلى انخفاض معنوي ($P<0.01$) في أنشطة الإنزيمات ALT، AST وALP وتركيزات اليوريا والكرياتينين و K^+، وارتفاع معنوي ($P<0.01$) في تركيز Na^+ بالمقارنة مع مجموعة CCl_4. وكذلك أدت معالجة الفئران بمستخلص الكركم والفلفل الأسود بالإضافة إلى CCl_4 إلى تحسن في التركيب النسيجي للكبد والكلى بالمقارنة مع المجموعات المعالجة بـ CCl_4.

نستنتج أن CCl_4 لـه آثـار ضـارة علـى الكبـد والكلـى وقـد أدى تنـاول مسـتخلص الكـركم والفلفل الأسـود إلـى حمايـة الكبـد والكلـى مـن هـذه الآثـار. لـذلك، ينصـح الأشـخاص المعرضـين للعوامل التي تسبب التسمم الكبدي والكلوي بتناول هذه المستخلص.

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I am extremely grateful to my parents for their love, prayers, caring and sacrifices for educating and preparing me for my future. I am very grateful to my husband who supported me and helped me in all stages of my studies.

I would like to extend my sincere thanks to the Medical Research Centre in Al-Zawia, and in particular to Dr. Akram for helping me to work in the animal house at the center. I also thank the Faculty of Medical Technology Surman, University of Sabratha in terms of welcome and permission work in the histopathology lab, and special thanks to Dr. Youssef Al-Ati for his cooperation.

Finally, I would like to thank everyone who helped and guided me throughout the course of my studies, from my teachers to mention Dr. Al-Sadiq Al-Ghoul.

Dedication

This thesis is dedicated to:

My great parents

My dearest husband

My beloved kids: Miral, Wesam, and Basher

Finally this thesis is dedicated to the memory of my daughter(Awsema).

Declaration

I hereby declare that this thesis is my original effort which is entitled:

{ Hepato-renal Protective Effect of Antioxidants (Curcuma and Black Pepper) Against CCL_4 Induced Toxicity, in Male Albino Rats }

There is no part has been plagiarised. Also, I declare that all the material submitted in this work, which is not my own work has been identified with proper citation and referencing and that no material is included that has been submitted for any others.

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Abbreviations

CCl_4	Carbon tetrachloride
I.P	Intraperitoneal
ROS	Reactive oxygen species
CCl3	Trichloromethyl
BUN	Blood urea nitrogen
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
PCO_2	Partial pressure of carbon dioxide
MDA	Malondialdehyde
Cl_3COO^-	Trichloromethylperoxyl radical
CCl_3^-	Trichloromethyl radical
PUFA	Polyunsaturated fatty acids
GM	Gentamicin
H & E	Haematoxylin & Eosin

CHAPTER 1

INTRODUCTION

&

LITERATURE REVIEW

1. Introduction

The liver is a vital organ in the body, essential for life because it plays a major role in metabolism, including ridding the body of substances that would otherwise be injurious if allowed to accumulate, and excretion of xenobiotic from the body (Al-Kenanny *et al.,* 2012, Ademiluyi *et al.,* 2013, Galaly *et al.,* 2014, Azab *et al.,* 2016). Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions (Azab, 2014).

The kidney is a common target for toxic xenobiotic due to its capacity to extract and concentrate toxic substances by highly specialized cells and also, due to its large blood flow (Azab *et al*., 2014). Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin (Sundararajan *et al*., 2014). It is identified by estimating the biomarkers like serum creatinine and serum urea which are considered reliable markers (Cyril *et al*.,2016).

Carbon tetrachloride (CCl_4) was used for metal degreasing and as dry cleaning, fabric-spotting, and fire extinguisher fluids, grain fumigant and reaction medium (Adewole1 *et al.,* 2007). A number of reports clearly demonstrated that CCl_4 causes disorders in kidneys, liver, lungs, testis as well as in blood by generating free radicals (Ozturk *et al*., 2003, Adewole1 *et al.,* 2007).

Medicinal plants and herbs play an important role in the prevention and treatment of liver and kidney diseases. Curcumin (*Curcuma longa* L) as one of the naturally occurring dietary substances has been used since ancient times for promoting human health (Joe *et al.,* 2004), which is used widely as a spice and coloring agent in several foods (Tirkey *et al.,* 2005). It represents a class of anti-inflammatory and antioxidants reported to be a potent inhibitor of reactive oxygen species (ROS) formation (Venkatesan *et al.,* 2000, Biswas *et al.,* 2005).

Many physiological effects of black pepper (*Piper nigrum*), its extracts, or its major active principle, piperine, have been reported in recent decades. Piperine is a major ingredient of black pepper and long pepper, which are widely used as a spice and in Ayurvedic medicine. (Singh *et al*., 2014). Their major active constituent of piperine, possesses various pharmacological actions including antioxidant, anti-inflammatory, antihyperlipidimic activity (Vijayakumar, and Nalini, 2006, Singh *et al*., 2014, Kumar *et al*., 2007), and hepatoprotective activity (Matsuda *et al*., 2008, Singh *et al*., 2014).

According to our knowledge, the reported literatures on the hepatorenal protective effect of curcumin, and black pepper against CCl_4 hepato-renalotoxicity are still limited. Therefore, it has become a task to prevent hepatic and renal damage induced by CCl_4 by eliminating free radicals and prevent lipid peroxidation through the use of a natural antioxidant rich plant like curcumin and black pepper.

1.1 Liver

The liver is considered a vital organ in the body plays a major role in drug detoxification, metabolism, glycogen storage, and plasma protein synthesis. Moreover; it is ridding the body from substances that would otherwise be injurious if allowed to accumulate, and excretion of xenobiotics from the body (Al-Kenanny *et al.,* 2012, Ademiluyi*et al.,* 2013, Galaly *et al.,* 2014). Liver diseases are still a global health problem may be classified as inflammatory liver diseases (acute or chronic hepatitis), non-inflammatory diseases, or functional disorders of the liver called (hepatosis) and degenerative disorders resulting in liver fibrosis (cirrhosis). Unfortunately, treatments of choice for liver diseases are controversial because conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects (Abushofa, 2014). Nowadays, the numbers of patients with liver dysfunction increase due to the overwhelming usage of alcohol and drugs has paved the path for researchers in interest in herbal medicine, because there are only a few universally effective and available options for the treatment of common liver diseases, such as cirrhosis, fatty liver and chronic hepatitis (Rajaratnam *et al.,* 2014).

1.1.1 Anatomy of the liver:

The liver is the largest gland in the body located inferior to the diaphragm, filling most of the right hypochondriac and epigastric regions (Figure.1.1). It is a reddish brown gland weighing about 1.4 kg. The liver is grossly divided into two parts when viewed from above – a right and a left lobe - and four parts when viewed from below (left, right, caudate, and quadrate lobes) (Figure.1.2). It secretes bile for the process of digestion (Kenneth, 2020).

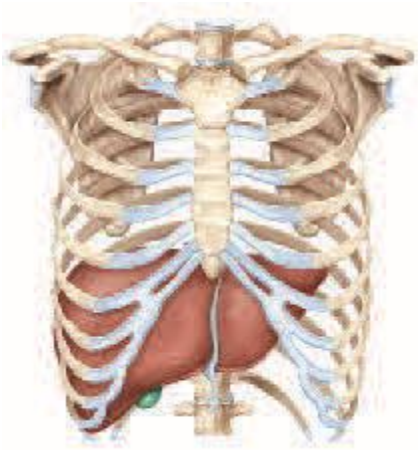


Figure.1.1: Location of the liver

(Kenneth, 2020).

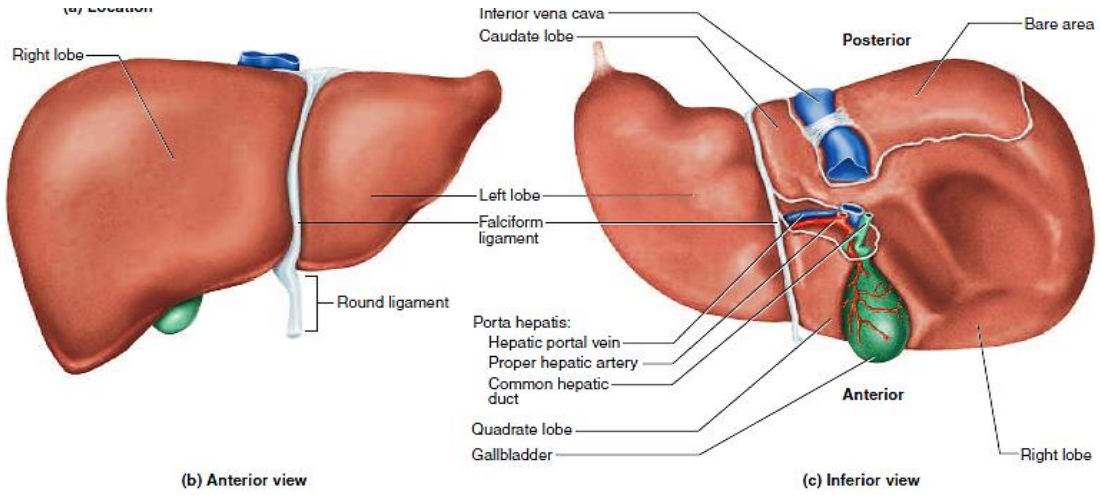


Figure.1.2: Gross anatomy of the liver

(Kenneth, 2020).

1.1.2 Structure of the liver:

Microscopically, each liver lobe is seen to be made up of hepatic lobules, which are the functional units of the liver. Each lobule consists of millions of plates of hepatic cells (hepatocytes) which are the basic metabolic cells. The sinusoids radiating from a central vein towards an imaginary perimeter of interlobular portal triads (Figure.1.3) (Jamal and Jamal, 2022).

One portal canal is located at each corner of the hexagonal classic lobule, making a total of six for each lobule (Figure.1.3& 1.4). These portal canals are composed of the portal triads, which are surrounded by loose stromal connective tissue. A periportal space (space of Mall), where lymph is produced, is sandwiched between the connective tissue of the portal canals and the hepatocytes. While connective tissue is present around the portal canals, the interlobular quantity is very small in humans. This can make routine histological visualizations of the classic lobule is difficult (Jamal and Jamal, 2022).

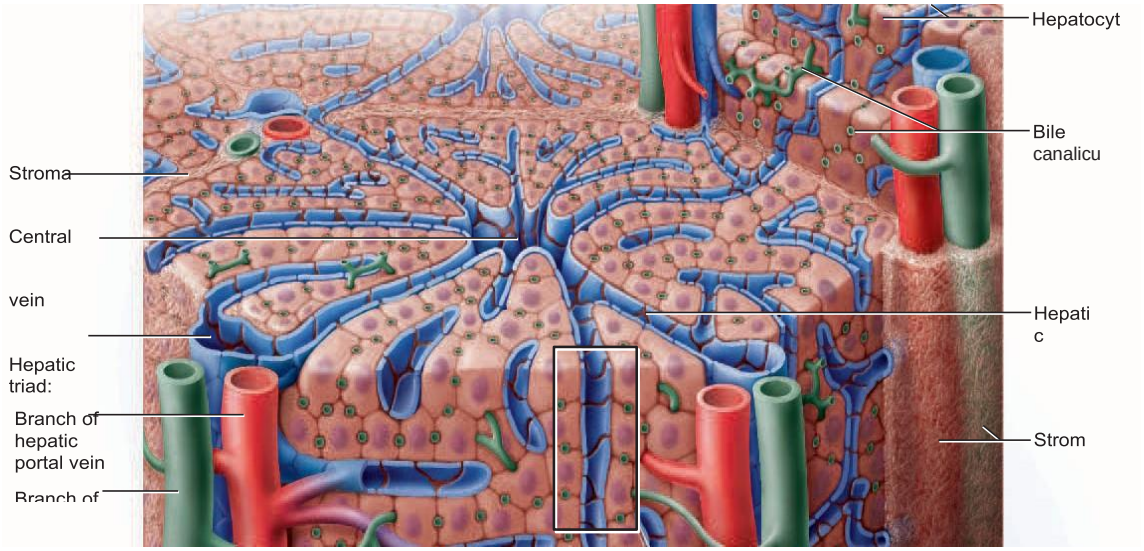


Figure.1.3: The hepatic lobules and their relationship to the blood vessels and bile tributaries (Kenneth, 2020).

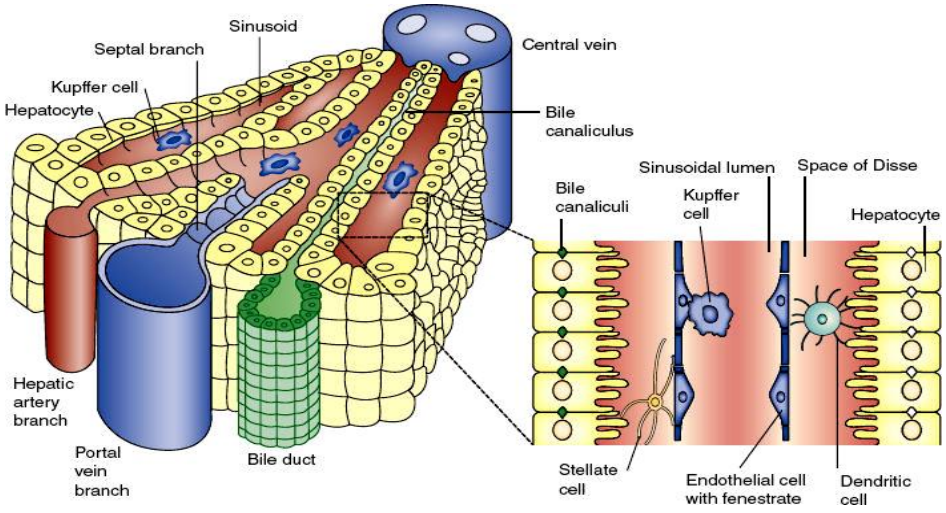


Figure.1.4: Portion of hepatic lobule: Microanatomy
(Adams, and Eksteen, 2006).

1.1.3. Functions of the Liver

The liver is an essential organ of the body that performs over 500 vital functions. These include removing bacterial bixoic, byproducts, toxins, and other harmful substances from the bloodstream, creating essential nutrients, regulating blood sugar levels, stores vitamins B_{12}, A, E, D, and K, as well as iron and copper, synthesis of most plasma proteins**,** and bile production (Mohan, 2005).

1.1.4. Metabolic zonation of the liver:

The classic lobule of the liver divided into a different metabolic activities area. The peripheral areas are called a zone of permanent (Z1), that have the highest blood supply, containing more oxygen metabolites. The middle zone of the lobules [zone of intermediate function (Z2)] has reduced metabolic activities. The most central zone is called the zone of permanent repose or inactivity (Z3), which closest to the central vein, and has the least metabolic function (Kietzmann, 2017) (Figure.1.5).

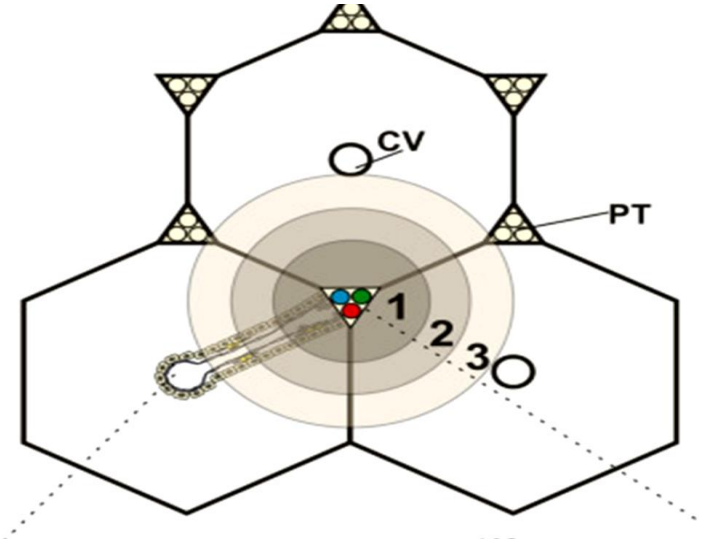


Figure.1.5: Liver microarchitecture, the oxygen gradient, and zonation of metabolism. Classic hexagonal-shaped liver lobule with a central vein (CV) in the middle and portal triad (PT) corners with a branch from the portal vein (blue dot), and a hepatic arteriole (red dot) as well as a bile duct (green dot). The acinus extends from a PT into the direction of two adjacent central veins. Three zones can be distinguished. 1, the periportal zone; 2, the intermediary zone; 3, the perivenous, pericentral, or centrilobular zone (Kietzmann, 2017).

1.1.5. Enzymes involved with the liver

1.1.5.1. Alanine transaminase

Alanine aminotransferase (ALT) is a transaminase enzyme which catalyzes the transfer of an amino group from alanine to α-ketoglutarate in the alanine cycle to form pyruvate and glutamate. It is found in serum and organ tissues, especially liver, and in a considerable amount in skeletal muscle, kidney, and heart. Also, ALT is present in low levels in spleen, pancreas, and lung. Serum ALT activity is used as a measure of liver function because it is significantly increased under conditions of cellular necrosis (Evans, 2009, Washington and Van Hoosier, 2012, Dhanya, 2020).

1.1.5.2. Aspartate transaminase

Aspartate transaminase (AST) is a transaminase enzyme containing pyridoxal phosphate. It catalysis reversible transfer of α-amino group between glutamate and aspartate. AST commonly present in liver, kidneys, skeletal muscle, brain, heart, and RBCs and it is commonly measured clinically as a marker for liver health (Nyblom *et al.,* 2006, Dhanya, 2020).

1.1.5.3. Alkaline phosphatase

Alkaline phosphatase (ALP) has functioning towards removing phosphate group containing molecules such as nucleotides, proteins, and alkaloids. It is mainly present in cells lining of the biliary ducts. Also, it is found in placenta and bone tissues and higher in growing children. The activity of blood ALP increases due to intrahepatic cholestasis and large bile duct constriction (Dhanya, 2020).

1.2. Kidney

The kidney is an organ that possesses several biological roles of which the most important is the homeostatic balance of body fluids by cleaning and secreting metabolites like urea, uric acid, creatinine, and minerals from the blood and excreting the nitrogenous wastes along with water, as urine (Javaid *et al*., 2012, Cyril *et al*., 2016). It maintains the overall chemical composition of the intracellular environment by regulating the quantity of water, sodium chloride, potassium, phosphate and numerous other substances in the body (Hoenig, and Zeidel, 2014, Mahipal, and Pawar, 2017). It is a common target for toxic xenobiotics due to its capacity to eradicate and concentrate toxic materials by highly specialized cells and also, due to its enormous blood flow (Choi *et al*., 2011, Azab *et al*., 2014).

Nephrotoxic effect is identified by estimating the biomarkers like serum creatinine and serum urea which are considered reliable markers (Cyril *et al*., 2016).

1.2.1. The structure of the kidney:

The kidney consists of an outer dark granular area called cortex and an inner pale area called medulla (Figure.1.6). Histologically, the kidney is composed of uriniferous tubules (the nephrons and the collecting tubules). The cortex zone contains renal corpuscles, convoluted tubules, peritubular capillaries, and medullary rays. The medulla zone is the inner pale area distingue, with radial striations due to the presence of loops of Henle, straight collecting tubules and vasa recta. The modularly rays or pars radiate are cortical tissue composed blood vessels and loops of Henle (straight segments of proximal and distal tubules and the straight collecting tubules). A renal lobe is formed of a medullary pyramid and the adjacent associated cortex.

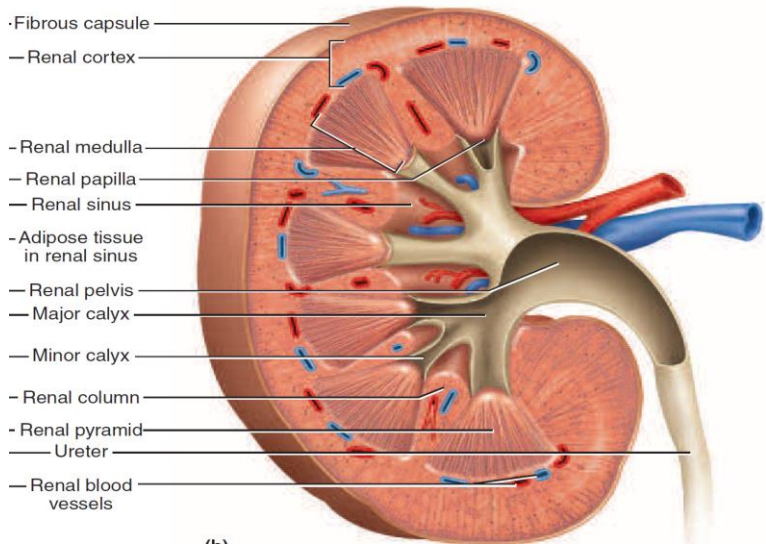


Figure.1.6: Gross Anatomy of the Kidney. Major anatomical features (Kenneth, 2020).

1.2.2. Nephron

Each kidney has about 1.2 million nephrons. The nephron is composed of two parts: a renal corpuscle, which filters the blood plasma, and a long coiled renal tubule, which converts the filtrate to urine (Figure.1.7) (Kenneth, 2020).

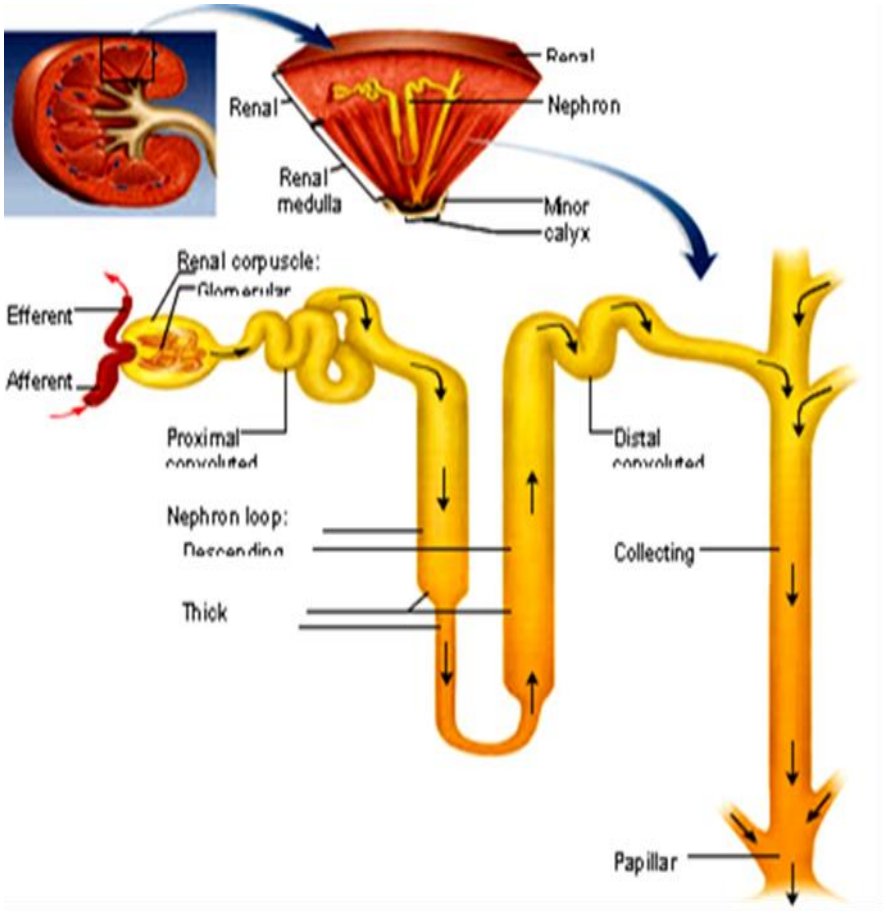
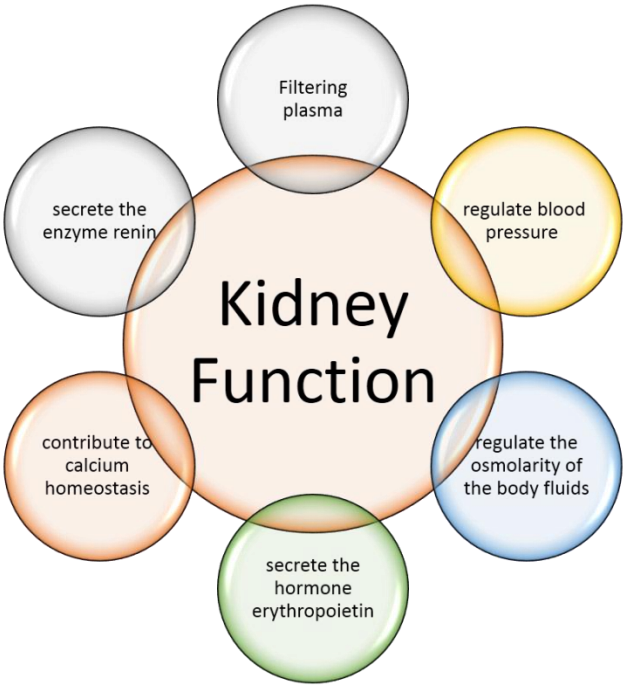


Figure.1. 7: Microscopic Anatomy of the Nephron

(Kenneth, 2020)

1.2.3. Functions of the Kidneys (Kenneth, 2020)



1.2.4. Kidney function markers

1.2.4.1. Blood Urea

Urea is produced by the breakdown of proteins and is excreted in the urine and blood urea is used to evaluate renal function in experimental animals. Blood urea level is affected by diet, hydration, circadian rhythm, and liver function (Melillo, 2007, Washington and Van Hoosier, 2012). The blood urea concentration is increased by a high-protein diet and forceful exercise, while it is decreased by liver failure, low protein intake, or treatment with anabolic steroids (Washington and Van Hoosier, 2012).

1. 2.4.2. Creatinine

Creatinine is a non-protein nitrogenous compound that is produced by the breakdown of creatine in muscle. It’s found in plasma, serum, and urine and is an indicator of renal function than blood urea because it is less influenced by hydration, and diet. It’s increased by lacking of glomerular filtration and necrosis or atrophy of hyperthyroidism, skeletal

muscle, burns, infections, and fractures (Evans, 2009, Washington and Van Hoosier, 2012).

1.3. Carbon tetrachloride

Carbon tetrachloride (CCl_4) (Figure.1.8) does not create naturally, it is a clear liquid with sweet smell that can be detected at low levels (Doherty, 2000, Adewole1 *et al.*, 2007). Volatile organic compounds such as CCl_4 are a class of solvents to which many people are exposed occupationally and environmentally. CCl_4 was formerly used for metal degreasing and as dry cleaning, fabric-spotting, and fire extinguisher fluids, grain fumigant and reaction medium. Because of its harmful effects, these uses are now banned and it is only used in some industrial applications. The primary routes of potential human exposure to CCl_4 are inhalation, ingestion, and dermal contact (Adewole1 *et al.*, 2007).

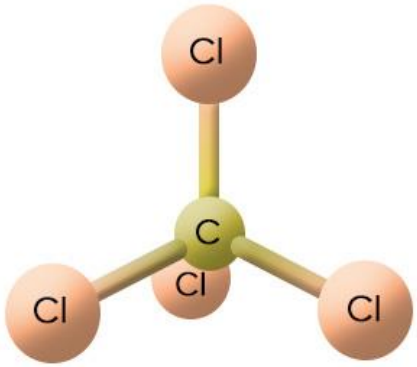


Figure.1. 8: Structural formula of carbon tetrachloride
(https://www.vedantu.com)

A number of reports clearly demonstrated that CCl_4 causes disorders in kidneys, liver, lungs, and testis as well as in blood by generating free radicals (Ozturk *et al*., 2003, Adewole1 *et al.*, 2007). A number of endogenous and exogenous nephropathy risk factors generate oxygen free radicals *in vivo*. Therefore, the role of oxygen derived free radicals and lipid peroxidation has attracted considerable attention

(Gebhardt, 2002, Das *et al*., 2005, Adewole1 *et al.*, 2007). Metabolism of CCl_4 involves in the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum. High exposure to CCl_4 can cause kidney damage. CCl_4 induces oxidative stress in many settings; therefore, it might be expected to contribute to nephrotoxicity. CCl_4 is one of such widely used environmental toxicant to experimentally induce animal models of acute nephrotoxicity and hepatic damages (Adewole1 *et al.*, 2007).

1.4. Oxidative stress:

The oxygen reactive species (ROS) generates from endogenous and exogenous sources. The endogenous generation of these species by inflammation mechanisms and activation of immune cells, cancerous, sever exercise, mental activity stress, infectious diseases, and aging. Exogenous sources of ROS result from the pollution of water and air, smoking, alcohol drinking, radiations, heavy metals, certain drugs (tacrolimus and cyclosporine), and some solvents as benzene. These compounds are decomposed into ROS after they penetrate the body (Valko *et al.*, 2007). ROS causes a damage to lipids, proteins, and nucleic acids. The formation of these free radicals leads to the initiation and progression of many diseases such as atherosclerosis, heart diseases, diabetes, cancers and liver diseases (Halliwell, 2007).

1.5. Medicinal plants

Medicinal plants and herbs play an important role in the prevention and treatment of kidney diseases. Curcumin (*Curcuma longa* L) as one of the naturally occurring dietary substances has been used since ancient times for promoting human health (Joe *et al*., 2004). Curcumin is major yellow pigments in rhizomes of *Curcuma longa* Linn which is used widely as a spice and coloring agent in several foods (Tirkey *et al*., 2005).

It represents a class of anti-inflammatory and anti-oxidant reported to be a potent inhibitor of reactive oxygen species (ROS) formation (Venkatesan *et al*., 2000).

Manikandan *et al*., 2011 and Azab *et al*., 2014 reported that curcumin affords curative role against nephro-toxicity induced gentamicin exposure and reduces gentamicin induced renal injury. Biswas *et al.*, 2005 found that curcumin has anti-inflammatory and antioxidant properties with a potent ability to inhibit reactive oxygen species formation.

Their major active constituent of piperine, possesses various pharmacological actions including antioxidant (Vijayakumar, and Nalini, 2006a, and Singh *et al*., 2014), anti-inflammatory (Kumar *et al*., 2007, Singh *et al*., 2014), and antihyperlipidemic activity (Vijayakumar, and Nalini, 2006b, Singh *et al*., 2014). Piperine is also known to exhibit a variety of biological activities which includes fertility enhancement (Singh *et al*., 2014), antioxidant activity (Jain and Mishra, 2011, Singh *et al*., 2014), antitumor activity (Manoharan *et al.*, 2009, Singh *et al*., 2014), hepatoprotective activity (Matsuda *et al.*, 2008, Singh *et al*., 2014).

The beneficial physiological effects of the black pepper (*Piper nigrum*) are incidentally attributable to their active principles Piperine (the biting principle of black pepper). Piperine is a major ingredient of black pepper and long pepper, which are widely used as a spice and in Ayurvedic medicine. Piperine {[1-5-(1, 3)-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl]-piperidine}, an alkaloid responsible for the pungency of black pepper & long pepper. Piperine {[1-5-(1, 3)-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl]-piperidine}, an alkaloid responsible for the pungency of black pepper. Black pepper is used not only in human dietaries but also for a variety of other purposes such as medicinal, as a preservative, and in perfumery. Many physiological effects of black

pepper, its extracts, or its major active principle, piperine, have been reported in recent decades (Singh *et al*., 2014).

Antioxidants such as melatonin, ascorbate, α- tocopherol, Silibinin, Lazaroid, Propionylcarnitine and superoxide dismutase/catalase, have been shown to ameliorate CCl_4-induced renal toxicity (Adewole1 *et al.*, 2007). Antioxidants play an important role in limiting and preventing the damage caused by reactive oxygen species (ROS). The hydroxyl radical possesses the highest one-electron reduction potential of all the physiologically relevant ROS and is extremely reactive with almost every type of biomolecule, including nucleic acids and proteins (Evans *et al.*, 2004).

1.6. The aims:

This study attempted to detect the effect of some antioxidants on the health of the liver and kidneys of rat models induced by carbon tetrachloride.

The general objectives of the study can be identified as follows

1- Investigate the harmful effects of CCl_4 on the liver and the kidney in male Fisher rats.

2- Evaluate the protective effects of Curcumin, and black pepper extract against the histopathological and biochemical alterations induced by CCl_4 in the liver and the kidney of male Fisher rats.

1-7 Literature Review

Dogukan *et al.*, (2003) reported that liver is the main target for CCl_4 toxicity while the kidney is the main site of CCl_4 accumulation. The pathogenesis of CCl_4-induced renal dysfunction is not completely understood.

Lee *et al.*, (2004) found that a single dose of CCl_4 (20 mg/kg) caused hepatotoxicity in the mice, as indicated by an increase in the ALT and AST serum levels after CCl_4 administration. Histopathological analysis showed that CCl_4 induced degeneration in the hepatocytes and hepatic cords as well as focal necrosis, as compared with the control.

Bhadauria *et al.*, (2008) demonstrated that oral administration of CCl_4 (0.5 ml/kg, p.o) to Female albino rats of *Sprague-Dawley* strain for 3 days significantly ($P < 0.01$) cause a significant increase in serum AST, ALT, and ALP activities, and serum urea and uric acid concentration.

Yang *et al.*, 2008 recorded that treatment of male Sprague-Dawley rats with a single oral dose of CCl_4 (1.25 ml/kg) caused severe hepatotoxicity, produced significantly elevated levels of serum AST and ALT activities. Histopathological examinations showed extensive liver injuries, characterized by extensive hepatocellular degeneration/necrosis, fatty changes, inflammatory cell infiltration, congestion, and sinusoidal dilatation.

Saad, (2012) reported that intraperitoneal injection of mice with a single i.p. dose of CCl_4 (20 mg/kg body weight) caused a marked liver cell necrosis with inflammatory and apoptotic lesions.

Saad, 2013 reported that the single CCl_4 dose (20 mg/kg body weight) is able to induce both hepatic injury and kidney dysfunction suggesting that the effect of CCl_4 on kidney function depends on the functional status of the liver. Hepatic injury can be induced by the administration of carbon tetrachloride via the production of free radicals.

Makni *et al.*, (2012) reported that CCl_4 (1ml/kg, intraperitoneally [i.p]) caused a significant induction of renal disorder, oxidative damage and DNA fragmentation as evidenced by increased plasma creatinine, urea and uric acid levels, increased lipid peroxidation (malondialdehyde [MDA]) and protein carbonyl. Furthermore, glutathione levels, catalase, superoxide dismutase, glutathione transferase and glutathione peroxidase activities were significantly decreased. Kidney histological sections showed glomerular hypertrophy and tubular dilatation in CCl_4-treated rats.

Adewole1 *et al.*, (2007) demonstrated that chronic administration of CCl_4 caused marked impairment in renal function alongside with significant oxidative stress in the kidney. Serum creatinine and blood urea nitrogen (BUN) concentrations were significantly higher in CCl_4-treated rats which are consistent with lower creatinine and BUN clearance. In addition, elevated level of urinary albumin and reduced level of serum albumin concentrations in CCl_4-treated rats might have resulted from remarkable leakage due to hypercellularity of both glomeruli and tubules. CCl_4 is metabolized by cytochrome P450 2E1 to trichloromethyl radical (CCl_3^-). CCl_3^- and its highly reactive derivative, the trichloromethylperoxyl radical (Cl_3COO^-), are assumed to initiate free radical-mediated lipid peroxidation leading to accumulation of lipid peroxidation products that causes renal injuries (Adewole1 *et al.*, 2007). These radicals are capable of initiating a chain of lipid peroxidation reactions by abstracting hydrogen from polyunsaturated fatty acids (PUFA). Peroxidation of lipids, particularly those containing PUFA, can dramatically change the properties of biological membranes, resulting in severe cell damage and play a significant role in pathogenesis of diseases (Adewole1 *et al.*, 2007). This phenomenon results in the generation of ROS, (like superoxide anion O^-, H_2O_2 and hydroxyl radical OH^-).

Evidence suggests that various enzymatic and non-enzymatic systems have been developed by mammalian cells to cope with ROS and other free radicals (Adewole1 *et al.,* 2007). However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient (Halliwell and Gutteridge, 2000, Adewole1 *et al.,* 2007). Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient; thus, reducing glomerular filtration rate (Garcia- Cohen *et al*., 2000, Adewole1 *et al.*, 2007).

Adewole1 *et al.,* (2007) found that histopathological alterations common to CCl_4-treated rats were glomerular hypercellularity, moderate to severe necrosis and tubulointerstitial alterations. It is believed that the capacity for tubular absorption may have been altered, thus bringing about functional overload of nephrons with subsequent renal dysfunctions.

Azab *et al.*, (2019) recorded that a significant ($P<0.01$) increase in serum urea, creatinine, and K^+ concentrations and a significant decrease in serum Na^+ concentration in rats treated with CCl_4 (1ml/kg of body weight) three time/week for 30 days as compared with the control group. Also, treatment of rats with CCl_4 resulted in a degeneration in epithelial lining the proximal convoluted tubules with disruption of their brush borders and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. Extensive interstitial hemorrhage was seen.

Azab *et al.*, (2016) reported that gentamicin induced hepatotoxicity was evidenced in Guinea pigs injected intraperitoneal with gentamicin at a dose of 100 mg/kg body weight/day by a significant increase in serum levels of AST, ALT, and ALP and a significant decrease in serum total

proteins, albumin, and globulin concentrations; and a significant alteration in hepatic architecture. Co-administration of Curcumin at the doses of 200 mg /kg body weight /day orally by gavage with GM for 10 days, prevented severe alterations of biochemical parameters and disruptions of liver structure, which may be due to its antioxidant property.

Biswas *et al.,* (2005) found that curcumin has anti-inflammatory and antioxidant properties with a potent ability to inhibit reactive oxygen species formation. Also, Kadasa *et al.,*2015 reported that curcumin significantly lowered the serum levels of ALT, and AST activities in rats treated with diethyl nitrosamine.

Ezz *et al.,* (2015) found that curcumin treatment to rats intoxicated with CCl_4 caused significantly reduced serum levels of ALT, AST and ALP activities, and significant elevations in serum total protein and albumin concentrations compared to CCl_4 intoxicated group. Treatment of curcumin reverted to the serum protein and albumin levels back to average in CCl_4-intoxicated rats, which reveals the well-functioning of hepatocytes in protein synthesis (Ezz *et al.,* 2015).

Manikandan *et al.,* (2011) and Azab *et al.,* (2014) were mentioned that the serum urea and creatinine were elevated in animals treated with gentamicin. Co-administration of curcumin with gentamicin caused a significant decrease in blood urea, and creatinine compared with gentamicin treated group.

Also, Azab *et al., (*2022) recorded that treatment of male albino rats with nicotine induced a significant increase in the serum creatinine and urea concentrations as compared with the control group. Concurrent adiminstration of rats with nicotine injection plus curcumin caused in a significant decrease in the serum creatinine and urea concentrations when compared with rats injected with nicotine group.

Sahu *et al* (2012) reported that in paracetamol induced hepatotoxic mice model, serum AST and ALT level demonstrated no significant elevation in the blood by the microspheres formulation. The histopathology and enzyme level results suggested that microsphere formulation can passively target hepatoprotective drug to the liver. Piperine inhibited increase in serum ALT and serum AST. The rate of inhibition depends on dose of piperine. It is suggested that inhibitory effect due to reduced sensitivity of hepatocytes to tumor necrosis factor (Singh *et al*., 2014).

Zhang *et al.*, (2021) mentioned that treatment of mice with CCl_4 caused a significant increase in serum ALT, AST, and ALP activities compared to the control group. Also, CCl_4 causes severe changes in the kidneys, specifically glomeruli, which appear small, shrunk, loosely arranged in Bowman capsules, and glomerular necrosis and vacuolarization and atrophy indicate tubular necrosis in the kidneys of mice after exposure to CCl_4. On the other hand, treatment of mice with CCl_4 plus the essential oil extracted from the black *Piper nigrum L*. caused a significant decrease in serum ALT, AST, and ALP activities compared to the CCl_4 treated group. Also, early supplementation with black pepper essential oil can return all indicators of kidney damage to almost normal levels, especially in the high-dose group.

Morsy *et al.*, (2020) reported that pretreatment of mice with piperine (50 mg/kg/day orally for 3 days) and injected Intraperitoneally with acetaminophen (650 mg/kg i.p. once) decreased serum alanine aminotransferase, decreased vascular congestion, cellular infiltration, as well as amelioration of hepatocellular necrosis, fat deposition, and pyknosis compared with acetaminophen overdose mice.

Begum *et al.*, (2015) recorded that intraperitoneal injection of rats with 100 mg/kg gentamicin for 8 days and orally received 50mg/kg

piperine from 1st day to 8th day caused a significant decrease in serum creatinine, BUN concentrations and improvemed the architecture of kidney tissues compared to the gentamicin treated group.

Sudjarwo *et al.,* (2017) reported that treatment of rats with lead acetate caused a significant increase in levels of BUN and creatinine when compared with the control group. Co-administration of piperine with lead acetate to rats resulted in a significant decrease in the levels of BUN and creatinine as compared with the lead acetate treated group. This might be through its direct action on free radicals of lead acetate, protecting the kidney from cellular damage by maintaining its membrane integrity.

CHAPTER 2

MATERIALS AND METHODS

2. Materials and Methods

2.1 Materials:

2.1.1 Animals:

In this study, 24 male adult Fischer rats weighing 200 ± 50 g were used. These animals were obtained from the animal house of the National Center for Medical Research in Zawia, Libya. Animals were placed in a room under standard conditions of ventilation, temperature, and relative humidity are hold at 25 ± 4 ^{O}C and 60 ± 5% respectively. Also, they were maintained on a normal cycle of light\dark. Rats were maintained on a standard lab diet and purified water with the addition of libitum. They were dealt with in the ethical framework and according to the rights of animal welfare.

2.1.2 Chemicals

Carbon tetrachloride (CCl_4) was obtained from the Chemistry Laboratory of the faculty of science, Al-Zawia university. Where, 0.5ml/kg body weight of CCl_4 was applied with (1:1: v: v) corn oil twice a week for four weeks.

Haematoxylin (H), and Eosin (E) stain were purchased from sigma –Aldrich, and pure paraffin wax (melting point 56^{O}C) was from faculty of Pharmacy - Tripoli University.

2.1.3 Preparation of curcumin and black pepper

Curcumin and black pepper were purchased from the local market and carefully washed under tap water, left to dry at room temperature, and were ground in a mixer and then weighed and mixed both curcumin and black pepper 3: 1 respectively, (300 mg curcumin, 100 mg black pepper) In 1 liter of corn oil. It was well mixed and put in a rolling shaker

for 24 hours to get it homogeneous and then filtered. The animals received 20 ml/kg/day of oil mixture daily by oral gavage for 28 days.

2.2 Methods

2.2.1. Experimental design:

Before the start of the experiment, the animals were left for a week for the purpose of adaptation. The animals were divided into 4 groups (Figure. 2.1) randomly after the weights of all animals were recorded. Each group of 6 rats was formed as follows:

Group I: (negative control) In the control group, animals were supplied with drinking water from the tap and a natural diet for 28 days.

Group II: (positive control) In the carbon tetrachloride group, rats were injected intraperitoneally with 1.5 mg/kg/day of diluted carbon tetrachloride (3 days a week) for 28 days.

Group III: Rats in this group were treated with curcumin and black pepper extract by a daily dosage for 28 days.

Group IV: (treated group) This group was treated with carbon tetrachloride intraperitoneally 3 days a week with daily curcumin and black pepper extract.

Figure. 2.1. The experimental animals in the animal house during the days of the experiment

2.2.2. Blood collection

At the end of the experiment and 24 hours after the last dose, all animals were anesthetized with, ether, and blood samples were collected by heart puncture. The blood samples were collected in a clean dry tube and centrifuged at 3000 rpm for 10 minutes then, the serum was separated and kept in a deep freezer at -20^{O}C until biochemical measurements carried out.

2.2.2.1 Measurement of serum liver enzymes:

Measurement of serum Alanine Aminotransferase (ALT) activity were used measured as a biomarker for liver injury as a result of the effects of some drugs (a marker of liver cell damage) serum ALT test used in performed in clinical chemistry section, Since the ALT is highly specific to the liver.

2.2.2.2 Measurement of serum urea, creatinine, and uric acid

Serum urea was measured based on the cleavage of urea with urease (Fawcett and Scott, 1960). Serum creatinine was measured without protein precipitation (Bartels *et al*., 1972). Sodium and potassium levels were estimated spectrophotometrically using commercial kits by the modified method described by (Maruna,1958).

2.2.3. Tissue collection

After killed the animals were dissected and a small part of the liver and kidney were fixed in formalin (10%) for 3 days and then were placed in a series of alcohol (dehydrate in an ascending series of alcohol), and that were passed on xylene, before embedded in paraffin wax at temperature (56 - 58 C°). All these steps were done by the automated tissue processor (Leica TP 1020-1-1).

2.2.3.2 Paraffin sections preparation

The samples were then submerged in paraffin wax in the shape of a mold by tissue embedding system (Leica EG1150H) and then took thicker sections (4.5 μm) on the microtome (RM2245; Leica Micro-systems), The tissue sections were then mounted on glass slides using a hot plate (HI1220; Leica Microsystems).

2.2.3.3 Hematoxylin and eosin staining protocol

The tissue sections were deparaffinised by xylene and rehydrated by different graded ethanol dilution (100%, 90%, and 70%). The sections were stained with haematoxylin and eosin (H&E). H & E staining was performed by the following protocol (Table. 2.1) for both liver and kidney tissues to study the histopathological changes under a light microscope and used colon for the validation of the H&E staining protocol. It is through consecutive steps as follows.

Table (2.1): H&E staining protocol

Step	Reagent	Time
1	Xylene X3	3 min
2	Ethanol 100,90,70,50%	3 min each
3	Hematoxylin	5min
4	Absolute Alcohol	1min
5	Tap Water	5 min
6	Purified Water	2 min
7	Eosin	3 min
8	Tap Water	5 min
9	Absolute Alcohol 50,70,80,90 %	1 min
10	Absolute Alcohol 100%	3 min
11	Xylene X2	5 min

2.3 Statistical analysis

All data is represented as mean ± standard deviation. Data were analyzed by a one-way analysis of variance (ANOVA). The difference between means ± SD was tested at $P<0.05$ using Duncan's multiple range test. This was done with GraphPad Prism 7.0 software. In all statistical tests, the probability level of $P<0.05$ was considered significant.

CHAPTER 3

RESULTS

3. Results

In the previous study in our Department of Zoology, my colleague studied the effect of the parsley plant on the kidneys of induced rats with carbon tetrachloride. In the current study, the effect of curcumin alone or with black pepper as an antioxidant was conducted on rats induced with carbon tetrachloride, and then the liver and kidney were investigated.

This study commenced from the preparation of a rat model exposed to carbon tetrachloride (CCl_4), which would damage body tissues, especially the liver and kidneys. Carbon tetrachloride is often used in animal experiments to study the effects of substances on liver injury and the related mechanisms of action, among which oxidative stress.

In this chapter, the potential positive effects of some antioxidants were studied, by examining the biochemical alterations of liver and kidney functions, and then confirming the results of the study through histological study.

3.1. Biochemical parameters

3.1.1 Biochemical parameters of liver results

3.1.1.1 Detection of the effectiveness of curcumin and black pepper on the liver enzymes in male albino rats after induced with CCl_4.

In general, the comparison with the control group, significantly increased serum levels of ALT, AST, ALP, were observed in the positive group ($P<0.05$), indicating that liver injury was successfully induced by CCl_4.

Serum activities of ALT, AST, and ALP of the different groups are presented in table 3.1 and figures (3.1- 3.3). Male albino rats were received intraperitoneal injection of CCl_4 only with 1.5 mg/kg of body

weight /day CCl_4 (3 days a week) for 28 days had significant (*P<0.01*) increased serum ALT, AST, and ALP activities (109.7 ± 6.53, 136.7 ± 7.45 & 200.2 ± 11.84) as compared with the control group (65.5 ± 2.07, 77.3 ± 2.34 & 105.8 ± 2.32), respectively.

In the treated groups, rats were injected intraperitoneal 3 days a week with 1.5 mg/kg of body weight/day carbon tetrachloride concurrently with 20 ml/kg/day of curcumin and black pepper extract daily by oral gavage for 28 consecutive days showed a significant (*P<0.01*) decrease in serum ALT, AST, and ALP activities (82.0 ± 3.85, 96.8 ± 2.48 & 125.3 ± 3.27) when compared with the CCl_4 group (109.7 ± 6.53, 136.7 ± 7.45, 200.2 ± 11.84), respectively (Table.3.1& Figures 3.1-3.3).

Table (3.1): Detection of the effectiveness of curcumin and black pepper on the liver enzymes in male albino rats after stimulated with CCl_4.

Groups	Control	Curcumin+ Black Pepper	CCl_4	CCl_4+ Curcumin+ Black Pepper
Parameters	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Serum ALT (IU/L)	65.5 ± 2.07	68.5 ± 2.66	109.7 ± 6.53**	82.0 ± 3.85
Serum AST (IU/L)	77.3 ± 2.34	82.0 ± 3.41	136.7 ± 7.45**	96.8 ± 2.48
Serum ALP (IU/L)	105.8 ± 2.32	111.7 ± 3.61	200.2 ± 11.84**	125.3 ± 3.27

**: Significant difference as compared to the control group at $p< 0.01$; Also, CCl_4 with antioxidants showed a significant difference compared to positive control group at $p< 0.01$.

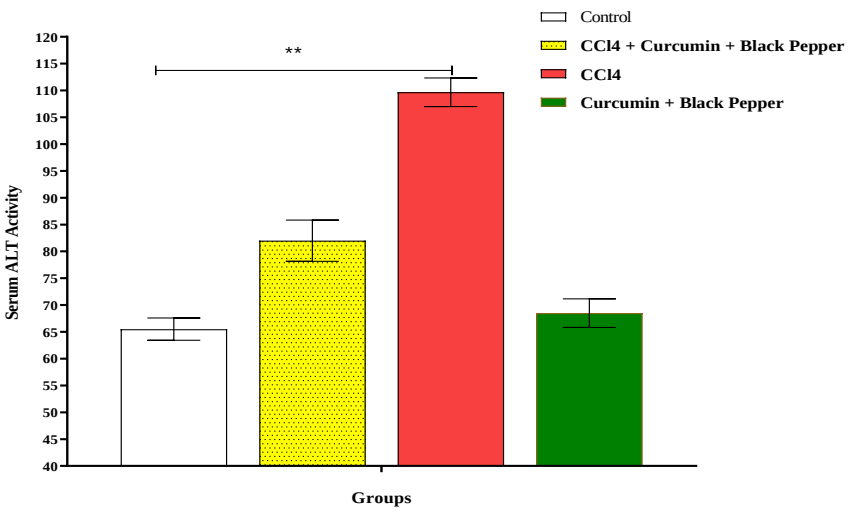


Figure 3.1: Effects of CCl_4, curcumin+ black pepper, and their combination on serum ALT activity of male albino rats. The bar chart showing significant elevate of serum ALT activity in the positive control group compared with the negative control group. Statistically significant difference from the control group is indicated by asterisks (P <0.0001).

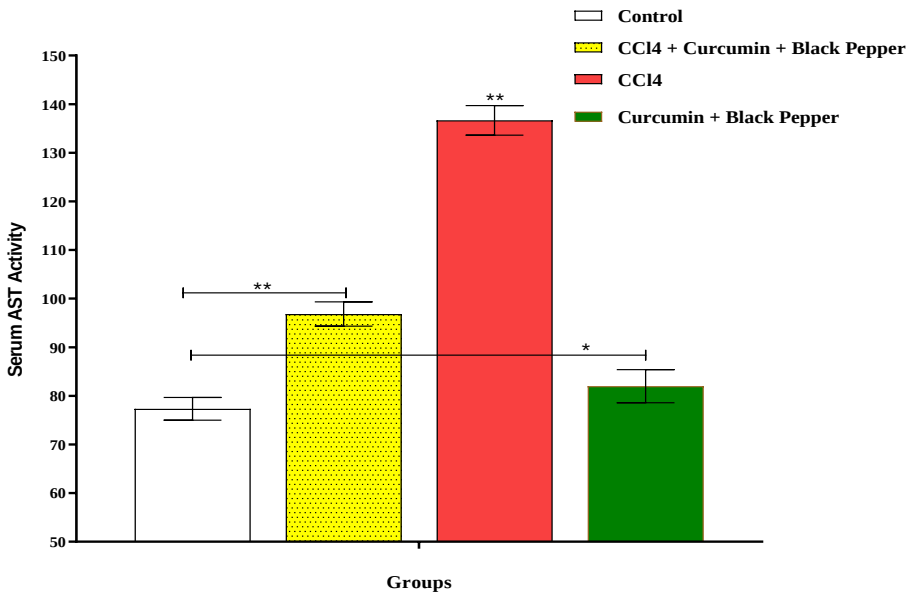


Figure 3.2: Effects of CCl_4, curcumin+ black pepper, and their combination on serum aspartate aminotransferase activity of male albino rats. The bar chart showing significant elevate of serum AST activities in the positive control group compared with the negative control group. Statistically significant difference from the control group is indicated by asterisks (P < 0.05).

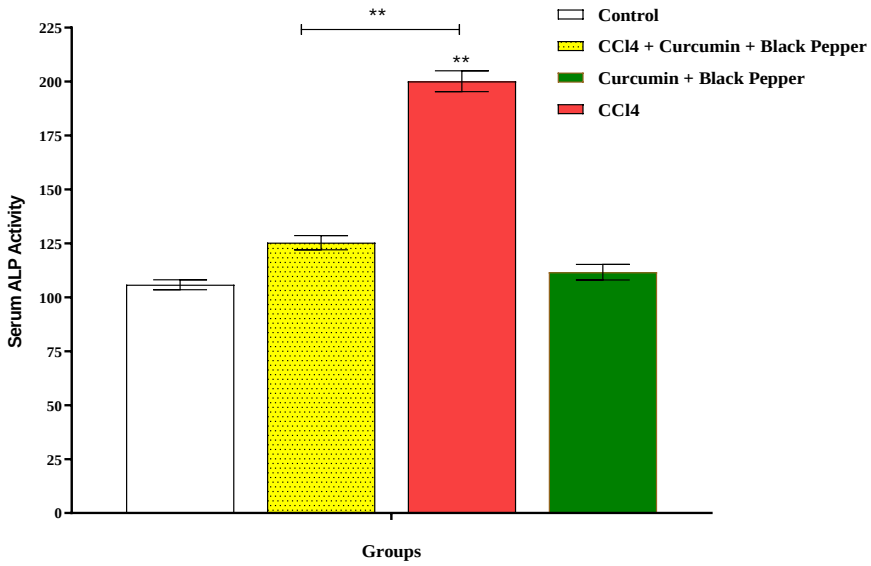


Figure 3.3: Effects of CCl_4, curcumin+ black pepper, and their combination on serum alkaline phosphatase activity of male albino rats. The bar chart showing significant elevate of serum ALP activities in the positive control group compared with the negative control group. Statistically significant difference from the control group is indicated by asterisks ($P < 0.0001$).

3.1.2 Biochemical parameters of kidney results

3.1.2.1 Detection of the effectiveness of curcumin and black pepper on the urea, creatinine, Na^+, and K^+ concentrations in male albino rats after stimulated with CCl_4.

Data in table 3.2 and figures (4-7) are shown serum urea, creatinine, Na^+, and K^+ concentrations of the different groups. Animals that received intraperitoneal injection of CCl_4 only with 1.5 mg/kg of body weight /day carbon tetrachloride (3 days a week)] for 28 days had a significant ($P<0.01$) increased serum urea, creatinine, and K^+ concentrations (55.5 ± 2.74, 1.2 ± 0.13 & 7.5 ± 0.95) when compared with the control group (34.0 ± 1.26, 0.6 ± 0.12 & 4.9 ± 0.16), respectively.

In contrast, the results of rats injected intraperitoneal 3 days a week with 0.5 ml/kg of body weight/day carbon tetrachloride for 28 days

showed a significant (*P<0.01*) decrease in Na^+ concentrations (126.8 ± 2.55) as compared with the control group (139.2 ± 1.50).

Simultaneous administration of 20 ml/kg/day of curcumin and black pepper extract to the group that was injected with CCl_4 daily for 28 days caused a significant (*P<0.01*) decrease in serum urea, creatinine, and K^+ concentrations (41.3 ± 1.51, 0.8 ± 0.14 & 5.1 ± 0.12) and a significant (*P<0.01*) increase in Na^+ concentrations (134.3 ± 1.63) when compared with the CCl_4 group (55.5 ± 2.74, 1.2 ± 0.13, 7.5 ± 0.95 & 126.8 ± 2.55), respectively (Table 3.2& Figures 3.4 -3.7).

Table (3.2): Effects of CCl_4, curcumin+ black pepper, and their combination on serum urea, creatinine, Na^+, and K^+ concentrations of male albino rats

Groups Parameters	Control	Curcumin+ Black Pepper	CCl_4	CCl_4+ Curcumin+ Black Pepper
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Serum Urea Concentration	34.0 ± 1.26	35.8 ± 2.32	55.5 ± 2.74**	41.3 ± 1.51
Serum Creatinine Concentration	0.6 ± 0.12	0.7 ± 0.12	1.2 ± 0.13**	0.8 ± 0.14
Na^+ Concentration	139.2 ± 1.50	138.9 ± 0.66	126.8 ± 2.55**	134.3 ± 1.63
K^+ Concentration	4.9 ± 0.16	5.0 ± 0.18	7.5 ± 0.95**	5.1 ± 0.12

**: Significant difference as compared to the positive control group at *p< 0.01*; Also, CCl_4 with antioxidants showed a Significant difference as compared to the positive control group at *p< 0.01*.

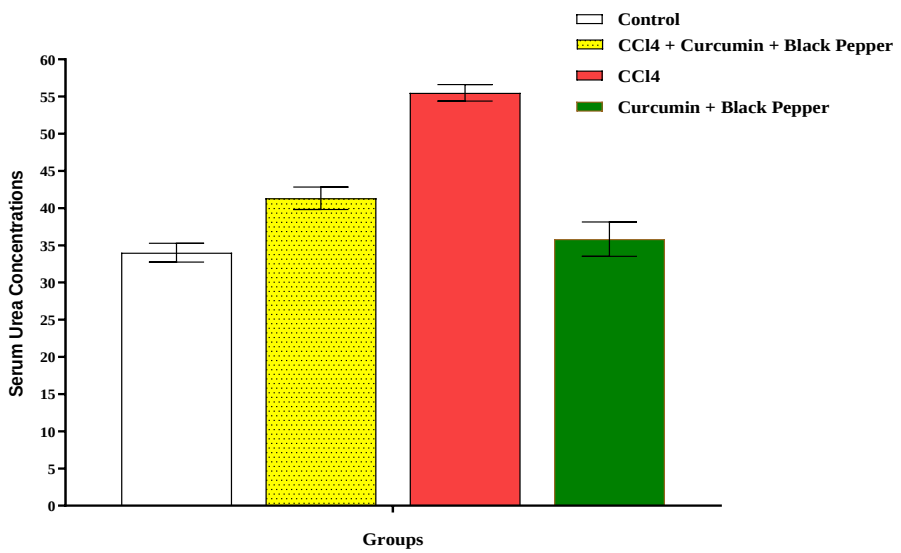


Figure 3.4: Effects of CCl_4, curcumin+ black pepper, and their combination on serum urea concentration of male albino rats. Bar chart showing significant elevate of serum urea concentrations in positive group compared with the treated group.

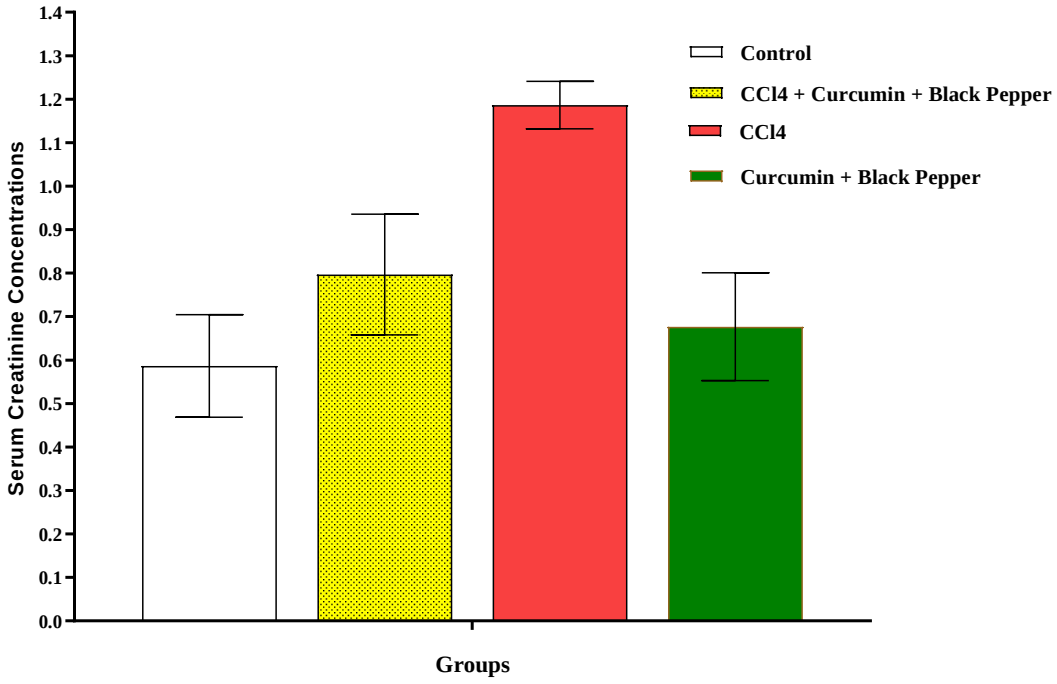


Figure 3.5: Effects of CCl_4, curcumin+ black pepper, and their combination on serum creatinine concentration of male albino rats. Bar chart showing significant elevate of serum creatinine concentrations in positive group compared with the treated group.

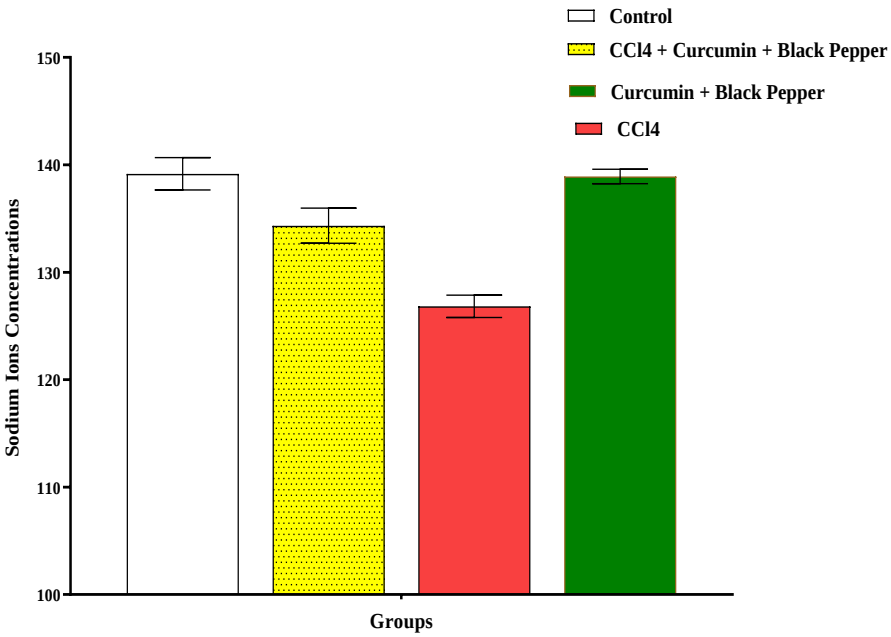


Figure 3.6: Effects of CCl4, curcumin+ black pepper, and their combination on serum sodium ions concentration of male albino rats. Bar chart showing significant elevate of sodium ions concentrations in positive group compared with the treated group.

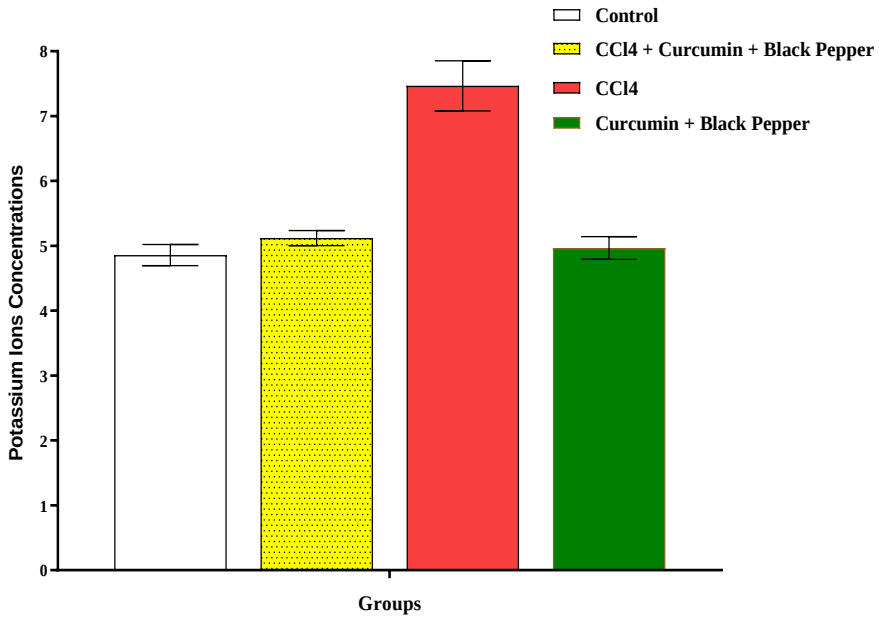


Figure 3.7: Effects of CCl4, curcumin+ black pepper, and their combination on serum potassium ions concentrations of male albino rats. Bar chart showing significant elevate of potassium ions concentrations in positive group compared with the treated group.

3.2. Histological examinations

3.2.1 Histopathological alterations of the liver

3.2.1.1 Liver sections of control rats:

In order to an emphasis the results which obtained from biochemistry parameters, it was important to confirm our results through histological sections and examine them by light microscopic. Before proceeding with staining the slides for the control and treatment groups, the validity of the stain was confirmed as a positive and negative control for the hematoxylin and eosin stains, the data not shown.

3.2.1.2 Liver sections representing negative control groups to verify the effect of curcumin and black pepper extracts:

The liver sections of curcumin and black pepper extract-negative control rats appeared with normal structures. The structure of the hepatic lobule appears normal. The hepatic cells are polyhedral in shape with large, centrally located nuclei and granular cytoplasm Figure 3.8 (A). The sinusoids are narrow blood spaces with irregular boundaries composed of endothelial cells in addition to large Kupffer cells and contain some RBCs Figure 3.8 (B).

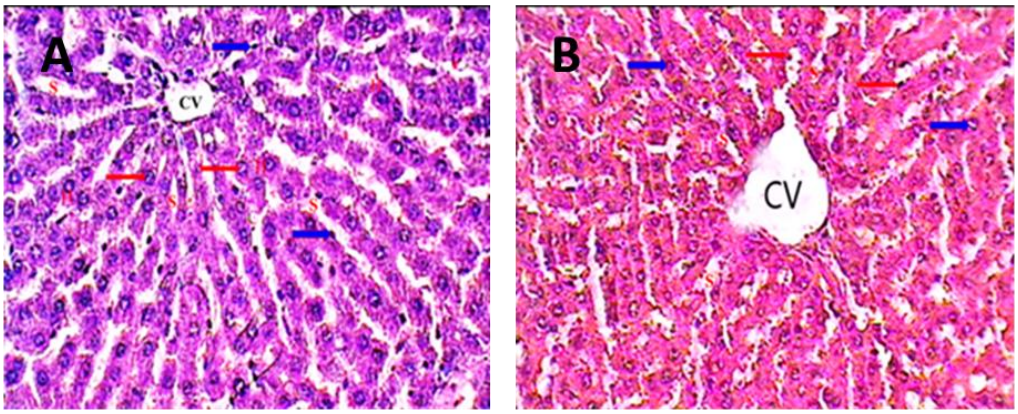


Figure 3.8 A: Light micrograph of section in the liver of the control rat. Central vein (CV); Hepatocyte (H); Blood sinusoid (S); Kupffer cells (Blue arrows); Nucleus (Red arrows) (Haematoxylin &Eosin ×100). B: Light micrograph of section in the liver of curcumin and black pepper extract-treated rat. Central vein (CV); Hepatocyte (H); Blood sinusoid (S); Kupffer cells (Blue arrows); Nucleus (Red arrows) (Haematoxylin &Eosin ×200).

3.2.1.3 Liver sections of rats induced with CCL_4:

The liver sections of CCL_4 group showed dilated congested central vein, damage in the lining endothelium, fibrosis in the wall of the central vein, hepatocytes are radially disorganized, and focal necrosis, some hepatocytes appear with vacuoles in cytoplasm, and congestion of some blood sinusoids contain activated kupffer cells (Figure. 3.9 B&C).

Also, induction of rats with CCL_4 caused a marked injury in portal area, leukocytic infiltration, and inflammatory cells around portal blood vessels, thick the wall of hepatic artery and bile ducts (Figure. 3.9 D).

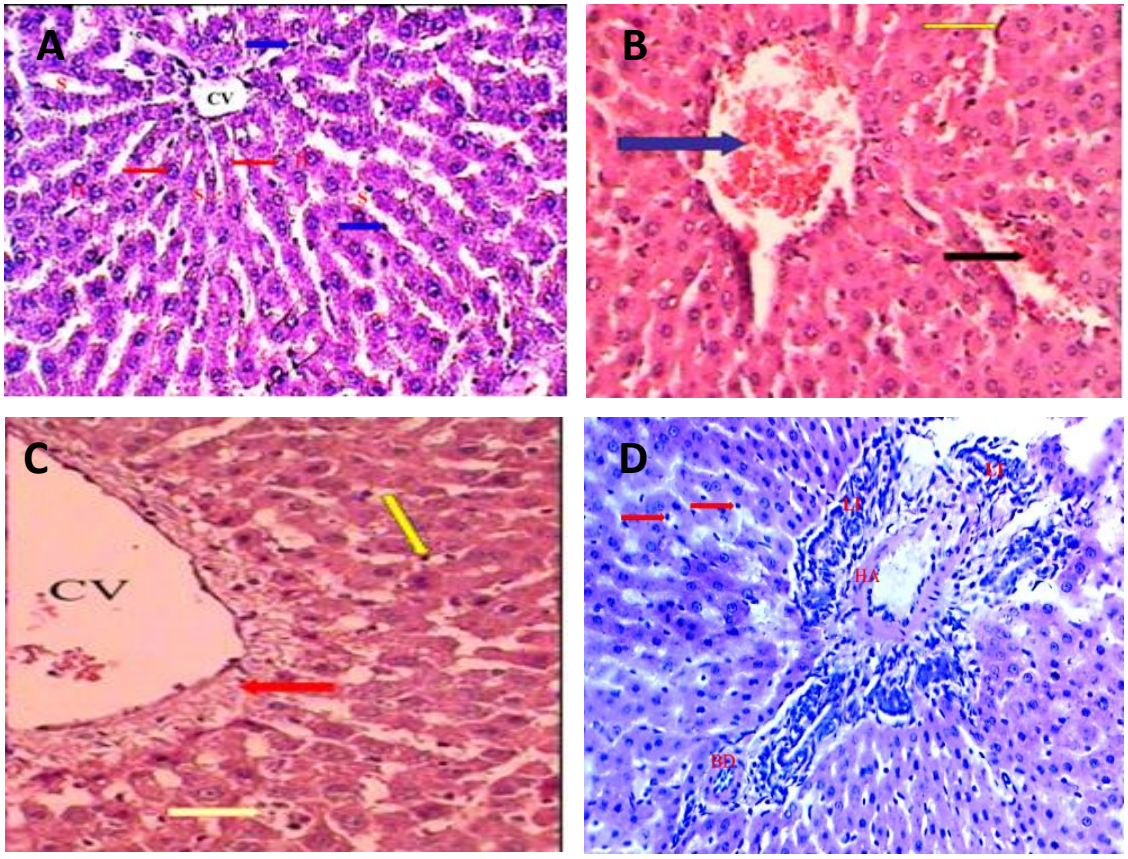


Figure 3.9: A: Light micrograph of section in the liver of the control rat. Central vein (CV); Hepatocyte (H); Blood sinusoid (S); Kupffer cells (Blue arrows); Nucleus (Red arrows). (B&C): Light micrograph of sections in the liver of the rat induced with CCl_4; Dilated central vein (CV); Congested central vein (Blue arrow); Dilated congested blood sinusoid (Black and white arrows); Activated Kupffer cells (Yellow arrows); Fibrosis (Red arrow). D: Light micrograph of section in the liver of the rat induced with CCl_4; Hepatic artery (HA); Leukocytic infiltration (LI); Activated Kupffer cells (Red arrows); Bile duct (BD) (Haematoxylin &Eosin ×200).

3.2.1.4. Liver sections of rats treated with curcumin and black pepper extract simultaneous CCl4.

Treatment of rats with curcumin and black pepper extract that had been induced with CCl_4 for 28 days caused improvement in the histological structure of the liver tissues. The structure of the hepatic lobules appeared normal. Some liver sections show dilated congested central veins, damage in lining endothelium, and dilated blood sinusoids with activated kupffer cells, (Figure. 3.10; B).

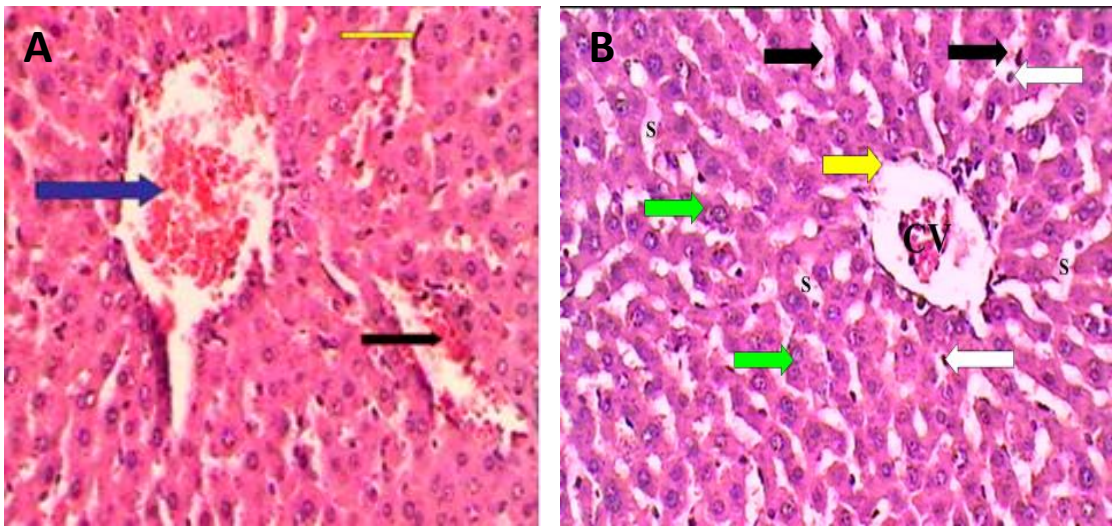


Figure 3.10: A; Light micrograph of sections in the liver of the rat induced with CCl4; Dilated central vein (CV); Congested central vein (Blue arrow); Dilated congested blood sinusoid (Black arrows); Activated Kupffer cells (Yellow arrows). B: Light micrograph of section in the liver of the rat treated with curcumin and black pepper extract plus CCl4 for 28 days. Congested central vein (CV); Damage in lining endothelium (Green arrow); Binucleated hepatocytes (Green arrows); Blood sinusoids (S); Activated Kupffer cells (White arrows); RBCs in sinusoids (Black arrows) (Haematoxylin &Eosin ×200).

3.2.2. Histopathological alterations of the Kidney

3.2.2.1. Kidney sections of control rats

The kidney sections of the control male albino rats group showed normal histological architecture. The renal cortex is formed of renal corpuscles (glomerular tuft of capillaries surrounded by Bowman's capsule), and normal proximal, and distal convoluted tubules. The proximal convoluted tubules are lined by a cuboidal epithelium with basally located spherical nuclei and a prominent brush border at the apical surface. The distal convoluted tubules are lined by cuboidal epithelial cells that lacked a brush border. (Figure. 3. 11).

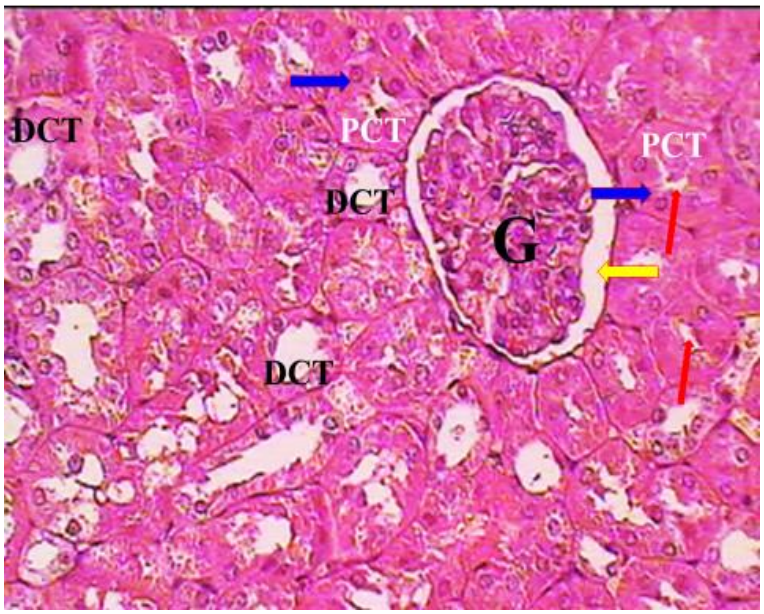


Figure 3.11: Light micrograph of Kidney section harvested from a control male albino rat; showed all structures of kidney are normal, Glomerulus (G); Bowman's capsule (Yellow arrow); Proximal convoluted tubules (PCT); Proximal convoluted tubule lumen (Red arrow); Distal convoluted tubules (DCT) (H&E ×200).

3.2.2.2. Histology alteration of the kidney:

The kidney sections of curcumin and black pepper extract-treated rats appeared with normal structures. The renal cortex was formed of renal corpuscles, and normal proximal, and distal convoluted tubules. The proximal convoluted tubules were lined by a cuboidal epithelium with basally located spherical nuclei, a prominent brush border at the apical surface, and the presence of epithelial debris inside the lumens of some proximal convoluted tubules. The distal convoluted tubules were lined by cuboidal epithelial cells that lacked a brush border. The nuclei were spherical and apically located with pale cytoplasm compared with proximal convoluted tubules (Figure. 3.12).

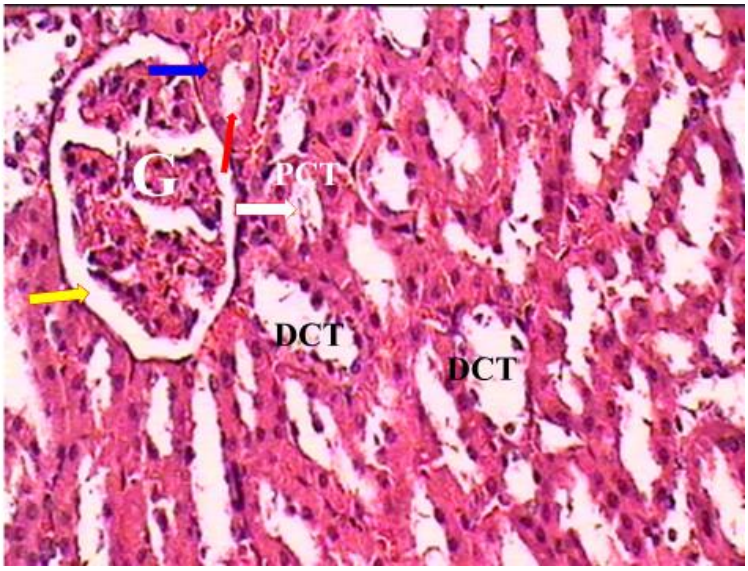


Figure 3.12: Light micrograph of the section in the Kidney of the curcumin and black pepper extract-treated rats; Glomerulus (G); Bowman's capsule (Yellow arrow); Proximal convoluted tubules (PCT); Proximal convoluted tubule lumen (Red arrow); Presence of epithelial debris inside the lumens of some proximal convoluted tubules (White arrow); Distal convoluted tubules (DCT) (H &E ×200).

3.2.2.3 Kidney sections of CCL_4 treated rats:

The kidney sections obtained from rats treated with CCl_4 show marked tissue damage. The proximal convoluted tubules show partial destruction of the brush border and desquamated cells were observed inside their lumens and the presence of epithelial debris inside their

lumens, some of which contained red blood cells. The cortex area of the kidney showed shrinkage of some glomeruli with wide capsular space, and congestion of blood vessels and interlobular spaces (Figure. 3.13 B-D).

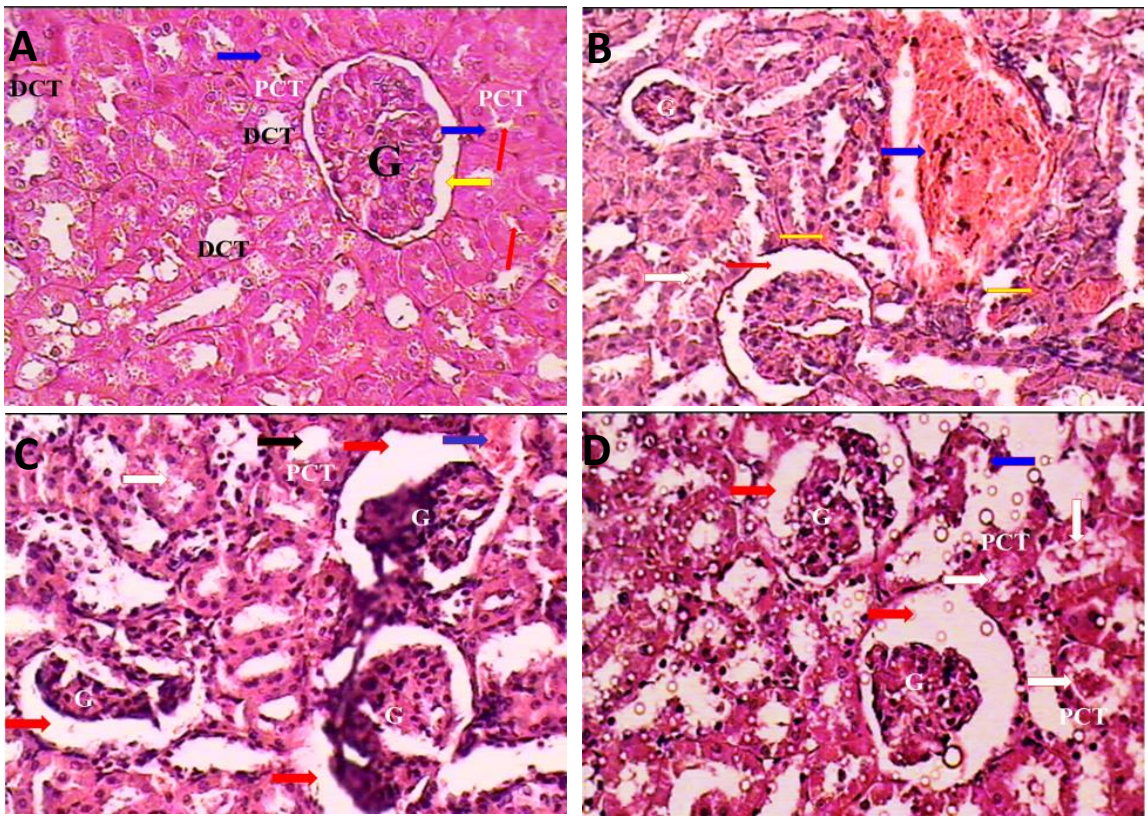


Figure 3.13: A: Light micrograph of the section in the Kidney of the control male albino rat; Glomerulus (G); Bowman's capsule (Yellow arrow); Proximal convoluted tubules (PCT); Proximal convoluted tubule lumen (Red arrow); Distal convoluted tubules (DCT). B-D: Light micrograph of the sections in the kidney of rat treated with CCl_4; Dilated Bowman's capsule (Red arrow); Presence of red blood cells between tubules (Yellow arrows); Dilated and congested blood vessel (Blue arrow); Shrinkage glomerulus (G); Presence of epithelial debris inside the lumen of proximal convoluted tubules (white arrow); Dilated lumen of proximal convoluted tubules (Black arrow); Degeneration of proximal convoluted tubules (Blue arrow in Fig. D). (H &E ×200).

3.2.2.4. Histopathological alterations of the kidneys in curcumin and black pepper treated groups.

Treatment of male albino rats with curcumin and black pepper extract after induced with CCl_4 for 28 days caused an improvement in the histological structure of the cortex of the kidney. The glomeruli were appeared less affected, markedly decreased the degree of injury and dilation of proximal convoluted tubules, tubules appeared normal except for the presence of few epithelial debris inside their lumens. Proximal convoluted tubules showed preserved brush border (Figure.3.14).

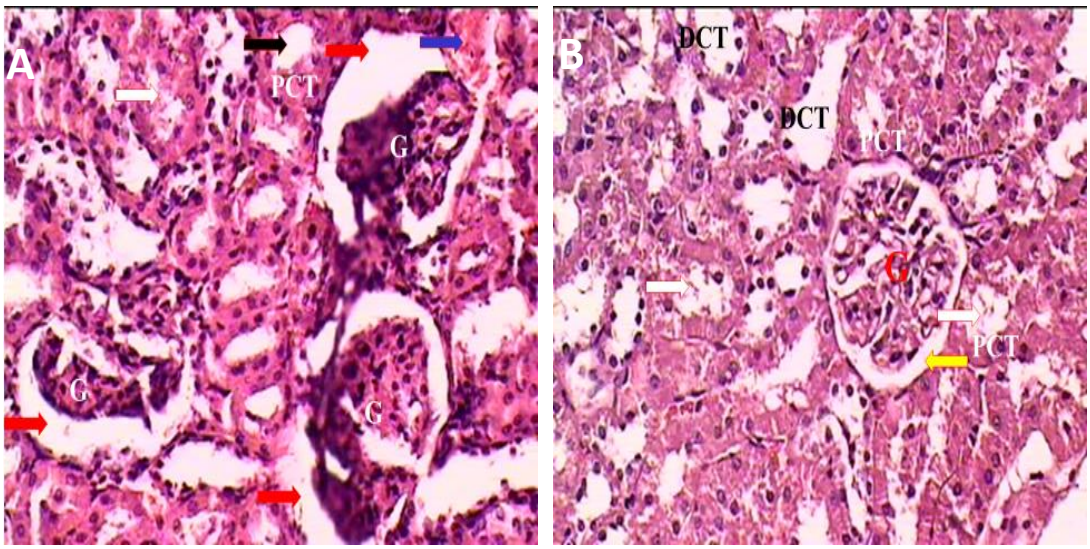


Figure 3.14: A; Light micrograph of the sections in the kidney of rat was induced with CCl_4; the slides showed dilated Bowman's capsule (Red arrow); dilated and congested blood vessel (Blue arrow); shrinkage glomerulus (G); dilated lumen of proximal convoluted tubules (Black arrow). B; a section of the Kidney was treated with curcumin and black pepper extract after induced with CCl_4. The slides showed; Glomerulus (G), Bowman's capsule (Yellow arrow), Proximal convoluted tubules (PCT), Presence of epithelial debris inside the lumens of some proximal convoluted tubules (White arrows), Distal convoluted tubules (DCT) (H &E ×200).

CHAPTER 4

DISCUSSION

5. Discussion

The liver is a primary organ of detoxification, and the major site of intense metabolism in general, thus undergoing to various disorders because its exposure to the toxins(RasGele & KaymaK, 2013). It was very importance to assess the liver enzymes serum that represent liver cells damage. It is common practice to use certain chemicals to induce hepatotoxicity in laboratory experiments, and then to study the toxicity, mechanism of liver functioning, and possible disorders. For these purposes, certain chemicals are used to induce hepatotoxicity (Abushofa *et al.,* 2019, Albasha *et al.*,2022).

The present study has investigated the protective effect of curcumin and black pepper extract against hepatorenal toxicity induced by CCl_4 in male albino rats.

Serum AST and ALT activities are known as toxicity markers in the hepatotoxicity studies. An increase in the activities of these enzymes is termed as the early recognition of toxic hepatitis (AL-Shinnawy, 2009, Albasha *et al.*,2022). It is known that, ALP is found in a high concentration in the bones and liver. Expectedly that serum ALP activity is increased in hepatobiliary diseases (Corathers, 2006) (Pike *et al.*, 2013), and during alcoholic hepatitis (Rukkumani *et al.*, 2003)

The present study showed that intraperitoneal injection of CCl_4 to male albino rats for 28 days had significantly ($P<0.01$) increased serum ALT, AST, and ALP activities as compared with the control group. Similar results were recorded by Zhang *et al.*, 2021 who found that induced of mice with CCl_4 resulted in a significant increased serum ALT, AST, and ALP activities compared to the control group.

The CCl_4 is metabolized by the cytochrome P-450 monooxygenase system to generate trichloromethyl radicals, and then reacts with oxygen to form trichloromethyl peroxy radicals (Shenoy *et al.*, 2001, Zhang *et*

al., 2021). These free radicals further attack cellular macromolecules, for instance proteins or lipids, bring about partial lipid peroxidation and cell necrosis of the liver, which results in the toxicity. The formation of lipid peroxides, such as MDA, leads to the loss of cell membrane integrity and damage to liver tissue (Zhang *et al.,* 2021). The important metabolic enzymes ALT, AST, and Alkaline phosphatase in liver cells are released into the blood when liver cells are injured (Azab *et al., 2019)*(Zhang *et al.,* 2021).

In the present work, the rats injected intraperitoneal with CCl_4 concurrently with curcumin and black pepper extract daily by oral gavage for 28 consecutive days showed a significant ($P<0.01$) decrease in serum ALT, AST, and ALP activities when compared with the CCl_4 group. These results run parallel with the study of Kadasa *et al.,* 2015 who reported that curcumin significantly lowered the serum levels of ALT, and AST activities in rats treated with diethyl nitrosamine. Also, Ezz *et al.,* 2015 recorded that co-administration of curcumin with CCl_4 to rats resulted in a significant decrease in serum levels of AST, ALT, and ALP activities compared with the CCl_4 treated group. In addition, co-administration of curcumin at the doses of 200 mg /kg body weight/day to Guinea pigs orally by gavage with gentamicin (100 mg/kg body weight/day) for 10 days, decreased serum levels of AST, ALT, and ALP and prevented the severe alterations in liver structure, which may be due to its antioxidant property (Li *et al.,* 2015).

Biswas *et al.,* (2005) reported that curcumin has antioxidant and anti-inflammatory properties for inhibiting reactive oxygen species formation. It could exert antioxidative effects either directly as a chemical antioxidant due to its ability to scavenge reactive oxygen and nitrogen free radicals or by modulating cellular defenses which themselves exert

antioxidant effects (Awasthi *et al.,* 2000, Priyadarsini *et al.,* 2003, (Hunyadi, 2019).

Similarly, piperine successfully restored liver function parameters in acetaminophen induced toxicity (Sabina *et al.,* 2010) and hyperlipidemia-induced hepatic steatosis in rats (Tunsophon and Chootip, 2016). Morsy *et al.,* 2020 found that pretreatment of mice with piperine (50 mg/kg/day orally for 3 days) and injected intraperitoneally with acetaminophen (650 mg/kg i.p. once) decreased serum ALT, improved liver microscopic structure and attenuated inflammatory and apoptotic markers. compared with acetaminophen treated group. Also, Zhang *et al.,* 2021 reported that treatment of mice with CCl_4 plus the essential oil extracted from the black *Piper nigrum L*. caused a significant decrease in serum ALT, AST, and ALP activities compared to the CCl_4 treated group. The essential oil extracted from the black *Piper nigrum L*. is rich in total phenolics, total flavonoids and proanthocyanidins, and showed good free radicals and lipid peroxidation scavenging capacities (Zhang *et al.,* 2021). The black *Piper nigrum* essential oil whereas considered has potential hepatoprotective positive effects (Jeena *et al.,* 2014; Butt *et al.,* 2013), The mechanism of action is most likely because of its antioxidant activity (Zhang *et al.,* 2021). Antioxidants are among the more effective molecules protecting against liver damage (Fraschini *et al.,* 2002).

Regarding the second aspect of this study, the possible renal disorders caused by toxicity by CCl_4. The reduction of the ability of kidney to eliminate the toxic metabolic substances is indicated by elevation of serum levels of creatinine and urea indications of kidney dysfunction (Hummadi, 2012, N. M. Kermani et al., 2021).

The current study showed that rats received intraperitoneal injection of CCl_4 for 28 days had a significant ($P<0.01$) increased serum urea, creatinine, and K^+ concentrations and a significant ($P<0.01$)

decrease in Na^+ concentrations when compared with the control group. These results are consistent with the results of previous studies (Makni *et al.,* 2012, Azab *et al.,* 2019). Azab *et al.,* (2019) recorded that a significant (*P<0.01*) increase in serum urea, creatinine, and K^+ concentrations and a significant decrease in serum Na^+ concentration in rats treated with CCl_4 (1ml/kg of body weight) three time/week for 30 days as compared with the control group. Makni *et al.,* 2012 found that intraperitoneally injection of rats with 1ml/kg body weight of CCl_4 resulted in a significant increase in plasma urea, and creatinine concentrations. Adewole *et al.,* 2007 reported that chronic administration of CCl_4 caused a significant increase in serum BUN concentrations and creatinine in CCl_4- treated rats.

CCl_4 is metabolized by cytochrome P450 to trichloromethyl radical (CCl_3^-). CCl_3^- and its highly reactive derivative, the trichloro-methylperoxyl radical (Cl_3COO^-), are assumed to initiate free radical-mediated lipid peroxidation leading to accumulation of lipid peroxidation products that causes renal injuries. These radicals are capable of initiating a chain of lipid peroxidation reactions by abstracting hydrogen from polyunsaturated fatty acids. Peroxidation of lipids, particularly those containing polyunsaturated fatty acids, can dramatically change the properties of biological membranes, resulting in severe cell damage and play a significant role in pathogenesis of diseases (Adewole *et al.,* 2007, (Abushofa, 2016). This phenomenon results in the generation of ROS, like superoxide anion O^-, H_2O_2 and hydroxyl radical (OH). Evidence suggests that various enzymatic and nonenzymatic systems have been developed by mammalian cells to cope with ROS and other free radicals (Adewole *et al.,* 2007, Azab *et al.,* 2019). However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient (Halliwell, and Gutteridge, 2000, Adewole *et al.,*

2007,(Abushofa et al., 2019). Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultra-filtration coefficient; and thus, reducing glomerular filtration rate (Garcia-Cohen *et al.,* 2000, Adewole *et al.,* 2007, Azab *et al.,* 2019). *In vitro* and *in vivo* studies indicate that CCl_4 enhances lipid peroxidation, reduces renal microsomal NADPH cytochrome P450, and renal reduced/oxidized glutathione ratio (GSH/GSSG) in kidney cortex as well as renal microsomes and mitochondria (Azab *et al.,* 2019).

In the present study, co-administration of rats with CCl_4 plus curcumin and black pepper extract for 28 consecutive days caused a significant (*P<0.01*) decrease in serum urea, creatinine, and K^+ concentrations, and a significant (*P<0.01*) increase in Na^+ concentrations when compared with the CCl_4 group. These results run parallel with that observed by previous studies (Manikandan *et al.,* 2011, Azab *et al.,* 2022). Manikandan *et al.,* 2011 and Azab *et al.,* 2014 were reported that the serum urea and creatinine were elevated in animals treated with gentamicin. Co-administration of curcumin with gentamicin caused a significant decrease in blood urea, and creatinine compared with gentamicin treated group. Also, Azab *et al.,* (2022) recorded that treatment of male albino rats with curcumin concurrent with nicotine injection resulted in a significant decrease in the serum creatinine and urea concentrations when compared with rats injected with nicotine group.

Likewise, Sudjarwo *et al.,* (2017) reported that co-administration of piperine with lead acetate to rats resulted in a significant decrease in the levels of BUN and creatinine as compared with the lead acetate treated group. This might be through its direct action on free radicals of lead acetate, protecting the kidney from cellular damage by maintaining

its membrane integrity. In addition, Begum *et al.,* 2015 reported that rats injected intraperitoneally with 100 mg/kg gentamicin for 8 days and orally received 50mg/kg piperine from 1st day to 8th day caused a significant decrease in serum creatinine, and blood urea nitrogen concentrations compared to the gentamicin treated group.

The current study included a histological study of hepatorenal sections to confirm the results of biochemical measurements, the liver sections in CCL_4 treated group show dilated congested central vein, damage in the lining endothelium, fibrosis in the wall of the central vein, disorganized, and focal necrosis, some hepatocytes appear with vacuoles in cytoplasm, and congestion of some blood sinusoids contain activated kupffer cells (Azab et al., 2019). treatment of rats with CCL_4 caused a marked injury in portal area, leukocytic infiltration, and inflammatory cells around portal blood vessels, thick the wall of hepatic artery and bile ducts. These hepatic structural alterations were paralleled to the changes obtained by Bahashwan *et al.,* (2015) demonstrated that treatment rats CCl_4 for two weeks showed pale stained hepatocytes around the central vein with cytoplasmic vacuolization, massive number of inflammatory cells infiltration, fatty change, degeneration, necrosis and apoptosis in most of the hepatic parenchyma. Also, Al-Jawad *et al.,* (2017) who reported that rabbit treated with CCl_4 showed a total loss of hepatic architecture with massive fatty changes, intense necrosis, congestion of sinusoids and infiltration of the lymphocytes around the central vein.

The current study, the treatment of rats with curcumin and black pepper extract plus CCl_4 for 28 days caused improvement in the histological structure of the liver tissues. The structure of the hepatic lobules appeared normal. Some liver sections show dilated congested central veins, damage in lining endothelium, and dilated blood sinusoids with activated kupffer cells. Similar results were reported by previous

studies (Balogun *et al.*, 2003, Sabina *et al.*, 2010, Azab and Albasha, 2018, Morsy *et al.*, 2020). Curcumin administration has been reported to prevent hepatic lesions in streptotocin diabetic rats and to protect against oxidative stress in hepatic cell lines (Balogun *et al.*, 2003, Choi *et al.*, 2013, Azab and Albasha, 2018, Abdel-Daim *et al.*, 2019, Morsy *et al.*, 2020).

Also, Morsy *et al.*, (2020) reported that mice receiving piperine with acetaminophen overdose decreased vascular congestion, cellular infiltration, as well as amelioration of hepatocellular necrosis, fat deposition, and pyknosis compared with acetaminophen overdose mice. Also, piperine improved the hepatic histopathology in mice treated with acetaminophen (Sabina *et al.*, 2010).

The antioxidant effects of piperine are well-established in different models of cellular injury either *in vitro* or *in vivo*, including models of hepatic injury (Vijayakumar *et al.*, 2004, Choi *et al.*, 2013, Abdel-Daim *et al.*, 2019, Morsy *et al.*, 2020). The upregulation of antioxidant enzymes was establised as a mechanism delineating the hepatoprotective effects of piperine (Morsy *et al.*, 2020).

In terms of kidney sections in the present study, examination of the kidney sections obtained from rats treated with CCl_4 showed a partial destruction of the brush border and desquamated cells were observed inside lumens of the proximal convoluted tubules and the presence of epithelial debris inside their lumens, and some of which contained red blood cells. The cortex area of the kidney showed shrinkage of some glomeruli with wide capsular space, congestion of blood vessels and interlobular spaces. These results similar to those described in the study of Adewole *et al.*, 2007 who confirmed that treatment of rats with CCl_4 induced glomerular hypercellularity, moderate to severe necrosis and tubule-interstitial alterations. In addition, Makni *et al.*, 2012 reported that

the kidney of rats injected intraperitoneally with 1ml/kg body weight of CCl_4 showed glomerular hypertrophy and tubular dilatation. Also, Azab *et al.,* 2019 reported that giving rats with CCl_4 resulted in a degeneration in epithelial lining the proximal convoluted tubules with disruption of their brush borders and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. Extensive interstitial hemorrhage was seen.

In addition, Zhang *et al.,* (2021) reported that CCl_4 causes severe changes in the kidneys, specifically glomeruli, which appear small, shrunk loosely arranged in Bowman capsules. Also, glomerular necrosis and vacuolarization and atrophy indicate tubular necrosis in the kidneys of mice after exposure to CCl_4 (Ozturk *et al.,* 2003, Zhang *et al.,* 2021). These changes may be the result of lipid peroxidation and membrane structure destruction (Zhang *et al.,* 2021).

The present results showed that treatment of male albino rats with curcumin and black pepper extract plus CCl_4 for 28 days caused an improvement in the histological structure of the cortex of the kidney when compared with the cortex of the kidney of CCl_4 treated groups. The glomeruli were appeared less affected, markedly decreased the degree of injury and dilation of proximal convoluted tubules, tubules appeared normal except for the presence of few epithelial debris inside their lumens. Proximal convoluted tubules showed preserved brush border. These alterations are similar to the results obtained by Manikandan *et al.,* 2011, and Azab *et al.,* 2014 were confirmed that co-administration of curcumin with gentamicin significantly improved the structural changes in the kidney of animals compared with gentamicin treated group. Azab *et al.,* 2022 reported that the animals injected subcutaneous daily with 0.8 mg, nicotine/kg body weight concurrently with curcumin 20 mg/kg diet daily for 30 days were caused a significant improvement the structural

changes in the kidney when compared with nicotine group. This histological structure of the kidneys was nearly similar to that in the control groups.

Al Anany *et al.,* (2015) suggested that curcumin exerts protective effects by improving the antioxidant system, inhibiting the oxidative stress induced by nicotine. The previous studies reported that natural antioxidants strengthen the endogenous antioxidants defenses from reactive oxygen species and restore the optimal balance by neutralizing reactive species (Deore et al., 2019) (N. Kermani et al., 2020) , 2014, Fetouh, and Azab, 2014). Curcumin has anti-inflammatory and antioxidant properties with a potent ability to inhibit reactive oxygen species formation (Biswas *et al.,* 2005, Azab *et al.,* 2022). Curcumin represents a class of anti-inflammatory and anti-oxidant reported to be a potent inhibitor of reactive oxygen species formation (Venkatesan *et al.,* 2000, Azab *et al.,* 2022). The ameliorative effect of curcumin against nicotine induced renal toxicity may be due to decrease uremic toxin, nitric oxide production, and increasing radical-scavenging enzyme activity through scavenging reactive oxygen and nitrogen species and chelating redex-active transition metal ions (Azab *et al.,* 2022).

Piper longum and *Piper nigrum* consist of more than 90% piperine, these piperines are of great interest due to antioxidant (Yadala, and Viswanathswamy, 2016) and anti-inflammatory properties (Sudjarwo, 2005).

Sudjarwo *et al.,* (2017) reported that co-administration of piperine with lead acetate to rats significantly improved the kidney histopathology compared with the lead acetate treated group. This means that piperine minimized the toxic effect of lead acetate via its antioxidant activity. The antioxidant protective mechanism scavenges the free radicals and decreases the oxidative stress, which is responsible for kidney damage

and thus inhibits the lipid peroxidation. Authors concluded that piperine could be a potent natural herbal product exhibiting nephroprotective effect against lead acetate induced nephrotoxicity in rats. Also, Begum *et al.,* 2015 recorded that rats injected intraperitoneally with 100 mg/kg gentamicin for 8 days and orally received 50mg/kg piperine from 1st day to 8th day caused an improvement in the architecture of kidney tissues compared to the gentamicin treated group. In addition, Zhang *et al.,* 2021 reported that structural changes in the cortical area were caused by CCl_4 are manifested in the destruction or expansion of the renal tubules and atrophy of the glomeruli. Early supplementation with black pepper essential oil can return all indicators of kidney damage to almost normal levels, especially in the high-dose group.

Conclusions & Recommendations

Conclusion

According to the previous results, it can be concluded that:

Intraperitoneal injection of CCl_4 to male albino rats for 28 days had significantly (*P<0.01*) increased serum ALT, AST, and ALP activities as compared with the control group.

The rats injected intraperitoneal with CCl_4 concurrently with curcumin and black pepper extract daily by oral gavage for 28 consecutive days showed a significant (*P<0.01*) decrease in serum ALT, AST, and ALP activities when compared with the CCl_4 group.

Rats received intraperitoneal injection of CCl_4 for 28 days had a significant (*P<0.01*) increased serum urea, creatinine, and K+ concentrations and a significant (*P<0.01*) decrease in Na+ concentrations when compared with the control group.

The liver sections of CCL_4 treated group show dilated congested central vein, damage in the lining endothelium, fibrosis in the wall of the central vein, disorganized, and focal necrosis, some hepatocytes appear with vacuoles in cytoplasm, and congestion of some blood sinusoids contain activated kupffer cells. Also, treatment of rats with CCL_4 caused a marked injury in portal area, leukocytic infiltration, and inflammatory cells around portal blood vessels, thick the wall of hepatic artery and bile ducts.

Treatment of rats with curcumin and black pepper extract plus CCl_4 for 28 days caused improvement in the histological structure of the liver tissues. The structure of the hepatic lobules appeared normal. Some liver sections show dilated congested central veins, damage in lining endothelium, and dilated blood sinusoids with activated kupffer cells.

Examination of the kidney sections obtained from rats treated with CCl_4 showed a partial destruction of the brush border and desquamated cells were observed inside lumens of the proximal convoluted tubules and the presence of epithelial debris inside their lumens, and some of which contained red blood cells. The cortex area of the kidney showed shrinkage of some glomeruli with wide capsular space, and congestion of blood vessels and interlobular spaces.

Treatment of male albino rats with Curcumin and black pepper extract plus CCl_4 for 28 days caused an improvement in the histological structure of the cortex of the kidney when compared with the cortex of the kidney of CCl_4 treated groups. The glomeruli were appeared less affected, markedly decreased the degree of injury and dilation of proximal convoluted tubules, tubules appeared normal except for the presence of few epithelial debris inside their lumens. Proximal convoluted tubules showed preserved brush border.

Recommendations

The present study recommended the following:

1. Individuals must be advised to ignore exposure to CCl_4.
2. Individuals must be take curcumin and black pepper extract for protection from hepatorenal toxic agents
3. Further studies are necessary with different doses of CCl_4, other natural products, and experimental animals.
4. Further studies are necessary to elucidate exact mechanism of hepatorenal protection and potential usefulness of curcumin and black pepper as a protective agent against CCl_4 induced hepatorenal toxicity in clinical trials.

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