THE EFFECTS OF HYDROETHANOL EXTRACT OF *Chasmanthera dependens* ON LIVER FUNCTION TEST PARAMETERS IN CARBON TETRACHLORIDE (CCl_4)-INDUCED TOXICITY

BY

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OCTOBER, 2023

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCE, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF BACHELOR OF SCIENCE, B.SC DEGREE (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY

OCTOBER, 2023

CERTIFICATION

We the undersigned hereby certify that **EDEMELEN ANABEL (BMS1802354**) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

_____ _____

Dr. Kelly Oriakhi **(Date)**
(Project Supervisor)

_____ _____

Dr. F. E.Olumese **(Date)**
(Head of Department)

_____ _____

External Examiner **(Date)**

DEDICATION

I humbly dedicate this work to Almighty God who has given me the strength, wisdom, grace and understanding to complete this research work in good health. I also dedicate this work to my ever supportive parents Mr and Mrs Edemelen , My sister Mrs Udoh, and the department of Medical biochemistry.

ACKNOWLEDGEMENT

I would like to express my deepest appreciation to everyone who has contributed immensely to the success of this research work at every stage of its development.

I really appreciate the effort my lectures of the department of Medical biochemistry made in grooming me for this phase, from my 100l to my finals. I sincerely appreciate my project supervisor, Dr Kelly Oriakhi who guided me through this work, patiently and thoroughly. I also want to express my deepest gratitude to Dr Mrs Nkeirukha Eluehike and Dr Mrs Ikpomwonsa Eweka for their support and guidance and making this research work hitch free and less cumbersome. I also want to appreciate my course adviser Dr Aguebor-Ogie for guiding me throughout my years in school.

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TABLE OF CONTENTS

ABSTRACT

Chasmanthera dependens is a plant known for its medicinal purposes. Liver diseases, especially those induced by toxins such as CCl_4, continue to be a significant global health concern. This study was conducted to evaluate the effects of hydroethanol extract of *Chasmanthera dependens* on liver function test parameters in CCl_4- induced toxicity. In this experimental study,25 male Wistar rats were divided into five groups :Group 1 was a control group, Group 2 was a CCl_4-treated group,Group 3 was a silymarin treated group of 140mg/kg, Group 4 and 5 were *Chasmanthera dependens* treated groups receiving different doses of the extract (250 and 500 mg/kg body weight) along with CCl_4 induction. The rats were sacrificed after 2weeks of treatment and blood was collected to run the analysis. There was a significant increase ($p<0.05$) in liver function parameters total bilirubin, albumin, total protein, and in globulin concentration, there was no significant difference in conjugated biliburin concentration when compared to normal control group. The group receiving the highest dose of *Chasmanthera dependens* (500 mg/kg) showed an improvement in liver function parameters, total protein and albumin. In conclusion, the hydroethanol extract of *Chasmanthera dependens* exhibited hepatoprotective effects against CCl4-induced liver toxicity by preserving liver function test parameters particularly in terms of protein synthesis and maintaining blood protein levels. These findings suggest the potential therapeutic use of *Chasmanthera dependens* in protecting the liver from toxic insults and warrant further investigations into its underlying mechanisms of action.

CHAPTER ONE

INTRODUCTION

1.0. Background of study

The creation of necessary proteins, detoxification, and metabolism are all heavily reliant on the liver, an important organ. Acute liver failure can result from toxicants injuring it, which can then alter the liver's metabolic processes and damage it.(Zamora-Nava *et al*.,). It is involved in over five hundred metabolic reactions within the biological system. One of its primary functions is the detoxification of poisons or harmful substances.

However, liver damage can be caused by exposure of the liver to hepatotoxic substances such as carbon tetrachloride (CCl_4). CCl_4 is a potent hepatotoxic drug that has been used as a buffer for inflicting liver damage by increasing the inflammation process and recruitment of inflammatory cells. CCl_4's toxic action involves the excessive production of reactive oxygen species(ROS), which in turn causes liver damage. Nevertheless, liver damage can be attributed to exposure to hepatotoxic substances such as carbon tetrachloride (CCl_4). CCl_4 is a potent hepatotoxic drug that has been used as a buffer for inflicting liver damage by increasing the inflammation process and recruitment of inflammatory cells. Ccl_4 toxic action involves the excessive production of reactive oxygen species (ROS), which in turn results in liver damage (Khan *et al*., 2012).

Chasmanthera dependens is a west African Plant also known as "Ato-Oloriraun" in the Yoruba Land. It belongs to the family Menispermaceae. It is a plant traditionally used for its medicinal properties due to its diverse pharmacological properties including anti inflammatory, antioxidant and hepato-protective effects. Traditional medicine has been a very essential source of therapeutic agents and the search for potential hepato-protective remedies has intensified in

recent years. *Chasmanthera dependens* has shown potentials in ameliorating hepato-toxicity and also a potential candidate for liver protection.

Liver function tests are blood tests used to help diagnose and monitor liver disease or damage. This tests measures the levels of certain enzymes and proteins in the blood. Some of these tests measures how well the liver is performing its normal functions of producing protein and clearing bilirubin, a blood waste product. The liver function test assess the liver's overall health and functions by measuring specific biomarkers such as Total protein (TP), bilirubin, albumin and globulin. Changes in these biomarkers can indicate liver inflammation or damage.

The liver function test parameters will be evaluated to determine the extent of liver protection offered by the hydroethanol extract of *Chasmanthera dependens* against CCl_4 induced toxicity. The potential of *Chasmanthera dependens* to help reduce liver damage could have significant implications for the development of new and natural therapeutic agents for liver disorders.

1.1. Justification of study.

One of the strongest heap-toxins is carbon tetrachloride, which is why scientists frequently employ it in studies to assess hepatoprotective treatment. (Seifert *et al.*,1994).

This current study aims to investigate the potential hepato-protective effects of the hydroethanol extract of *Chasmanthera dependens* in a CCl_4-induced liver toxicity. Traditional plants are high in phytochemicals and they have therapeutic effects on both humans and animals and may be used to make drugs.

Different phytochemicals and antioxidants found in medicinal plants are used to cure a variety of illnesses, including malaria, diarrhea, tuberculosis, asthma, and others. Since the beginning of

time, medicinal plants have been used to treat human and animal illnesses (Osai, 1998; Ibewike *et al.,* 1997).

These results may not only contribute to scientific knowledge but also provide a basis for considering *Chasmanthera dependens* as a potential candidate for inclusion in traditional medicine or complementary therapy or liver related conditions.

1.2. Aim of study

The aim of this study is to investigate the effects of hydroethanol extracts of *Chasmanthera dependens* on liver function test on CCl_4 induced liver toxicity.

CHAPTER TWO

LITERATURE REVIEW

2.0. Chasmanthera dependens

From Sierra Leone's east to Eritrea and Somalia, and from Tanzania and the Eastern Democratic Republic of the Congo to Angola, Zambia, and Zimbabwe, *Chasmanthera dependens* is a widely dispersed plant. (Iwu *et al*.,1999).In Ghana, it is frequently planted in backyard gardens. It is a member of the Menispermaceae family. It is an angiosperm. Chasmanthera is its common name. It is also referred to as "Atoo-Oloriraun" in Nigeria's southwest.

Traditional healers make use of this woody climber, which can be found in secondary forests and forest borders, for treating malaria patients. (Iwu *et al*.,1999).The people of Ahiazu Mbaise, South East Nigeria, frequently use it to treat malaria and other illnesses.

In West Africa, People use the leaf and the stem sap as a local remedy for sprains, bruises and fractures. They also mix it with Shea butter to create an ointment that helps to relieve pain and stiffness. The bark of the plant is also taken orally to treat venereal discharge or also as a general tonic for physical or nervous weakness in inflammatory and exhausting diseases.

Mature branches of *Chasmanthera dependens* have papery exfoliating bark, while young branches are densely branched, short, and hairy. This plant has medicinal properties and is used to treat a variety of illnesses, such as infections of the red eyes (Ogunlesi *et al*., 2005), fractures and venereal diseases (Odugbemi, 2008), and neurological and physical disabilities (Iwu *et al*., 1999). It is said that the leaves' aqueous extract, when combined with local alcohol, has aphrodisiac properties. Additionally, it has anti-inflammatory, anti-arthritic, anti-allergic, anti-periodic, and anti-diabetic(Irungy.,2012)

Antimicrobial activities of *Chasmanthera dependens* stem on fungal yeast, Gram+ and Gram− bacteria were reported by (Githinji *et al.*,2010). In vitro, anti-leishmanial and immunomodulatory activities of *Chasmanthera dependens* stem and leaves were reported by (Iwu *et al.*,1999). Aqueous extract of *Chasmanthera dependens* roots demonstrated pro-spermatogenic, fertility-enhancing, and androgenic activities in male rats (Quadri and Yakubu 2017). Administration of exogenous substance such as *Chasmanthera dependens* requires the evaluation of serum levels of synthetic products of the liver such as total protein, albumin, bilirubin and globulin so as to identify any compromise of its functional capacity or any form of liver damage consequent to ingestion. (Quadri and Yakubu 2015).

Various Studies of this plant shows that it has anti-inflammatory and analgesic activities (Onabanjo *et al*.,1991), antifungal activity (Adekunle and Okoli, 2002), and antimicrobial l(Githinji *et al*.,2010; Ogunlensi *et al*., 2010).

Fig 2.1.*Chasmanthera dependens. THE GREEN INSTITUTE, 2020*

2.1. Phytochemicals of *Chasmanthera dependens*

According to an analysis, the stem bark of *Chasmanthera dependens* is rich in alkaloids. It contains the non-phenolic quaternary alkaloids tetrahydropalmatine, liriodenine, lysicamyine (oxonuceferine), o,o-dimethylcorytuberine, anonaine, glaucine, norglaucine, oxoglaucine, and nornuceferine. The stem bark of the plant is rich in alkaloids.

These chemicals, known as alkaloids, have been shown to have a number of therapeutic benefits, such as anti-leishmaniasis and anti-parasitic actions. It also contains the furanoid diterpene-8-hydroxy columbine, the pavine type alkaloid his norargemonine, the morph in andirons type alkaloid pallidine, and the tetrahydroprotoberberine type alkaloids govanine and coreximine. It was discovered that a number of these alkaloids had intriguing pharmacological effects after being extracted from various other species.

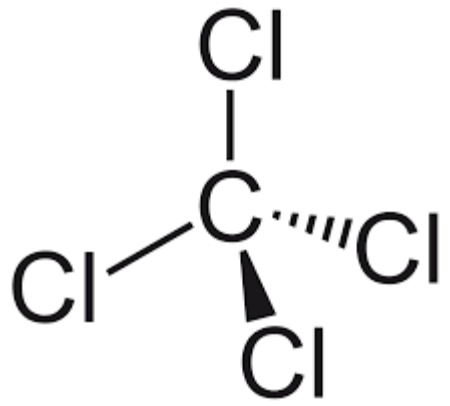
From earlier research, the roots ethanol extracts and crude water extract demonstrated strong antifungal activity against Candida albicans, Microsporum Audonii, Aspergillus fumigatus, Aspergillus Niger, Trichoderma viride, and Trichophyton Mentagrophytes. The plant extracts in ethanol demonstrated greater activity than the extract in water.

Chasmanthera dependens occurs most commonly in forest margins, savannah and secondary forests, often near rocks, but also in dense and moist evergreen forest, semi deciduous forest and riverine forests up to 1500 meters altitude.

These phytochemicals have been associated with potent antioxidant, anti-inflammatory, and hepatoprotective activities. The antioxidant properties are particularly relevant in the context of

liver protection, as they can scavenge free radicals and reduce oxidative stress-induced liver damage.

2.2 Carbon tetrachloride (CCl_4) Toxicity



Carbon tetrachloride is a significant agent for several purposes,It can be used as cleaning agents or even in industries as agents, but it can also cause several adverse effects in the human body.

The combination of chlorine and chloroform in the presence of light yields CCl_4, a colourless, volatile, non-flammable liquid. Tetrachloromethane, as it is structurally known, is a chlorinated hydrocarbon.

In the past years, CCl_4 was used as a cleaning agent and degreaser in homes, industrial manufacturing, dry cleaning textile laundries, in fire extinguishers and also used as a precursor of refrigerant's and propellants. Because of its highly toxic and harmful effects, some of its uses are presently banned. Although its use in some industries continues. Inhalation of its vapours, dermal absorption following direct skin in humans. Mostly in the liver, kidney, and lungs, CCl_4 damages cells in a number of organs.(Teschke ,2018), (Slater *et al*.,1985).

CCl_4 toxicity does not develop due to CCl_4 itself but develops due to the generation of free radicals CCl_3 and other metabolites produced by cytochrome p450 ultimately they lead to cellular damage by alteration of cellular structure through lipid per oxidation and in some other pathways. Severe conditions may develop through multiple organ dysfunction by these free radicals. (Manno *et al*.,1996).

2.3. Mechanism of action of CCl_4 on liver damage.

An established. CCl_4 is activated by cytochrome (CYP) 2E1, CYP2B1 or CYP2B2, and possibly CYP3A, to form the trichloromethyl radical, CCl_3−(Slater, 1984). This radical can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes such as lipid metabolism, which results in fatty degeneration (steatosis) (Raucy *et al*.,1993). CCl_3− forms adducts with DNA, which initiate the onset of hepatocellular carcinoma. This radical can also react with oxygen to form the trichloromethylperoxy radical CCl_3OO−, a highly reactive species. CCl_3OO− reacts with polyunsaturated fatty acids and phospholipids to initiates the chain reaction of lipid peroxidation. This affects the permeabilities of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage (Weber *et al*.,2003, Mehendale *et al*.,1994). Reactive aldehydes, particularly 4-hydroxynonenal, are among the byproducts of fatty acid degradation that attach readily to protein functional groups and obstruct vital enzyme functions (loss of glucose-6-phosphatase activation). (Boll *et al*.,2001).thereby leading to liver injury (Sevanian,Ursini.,2000).

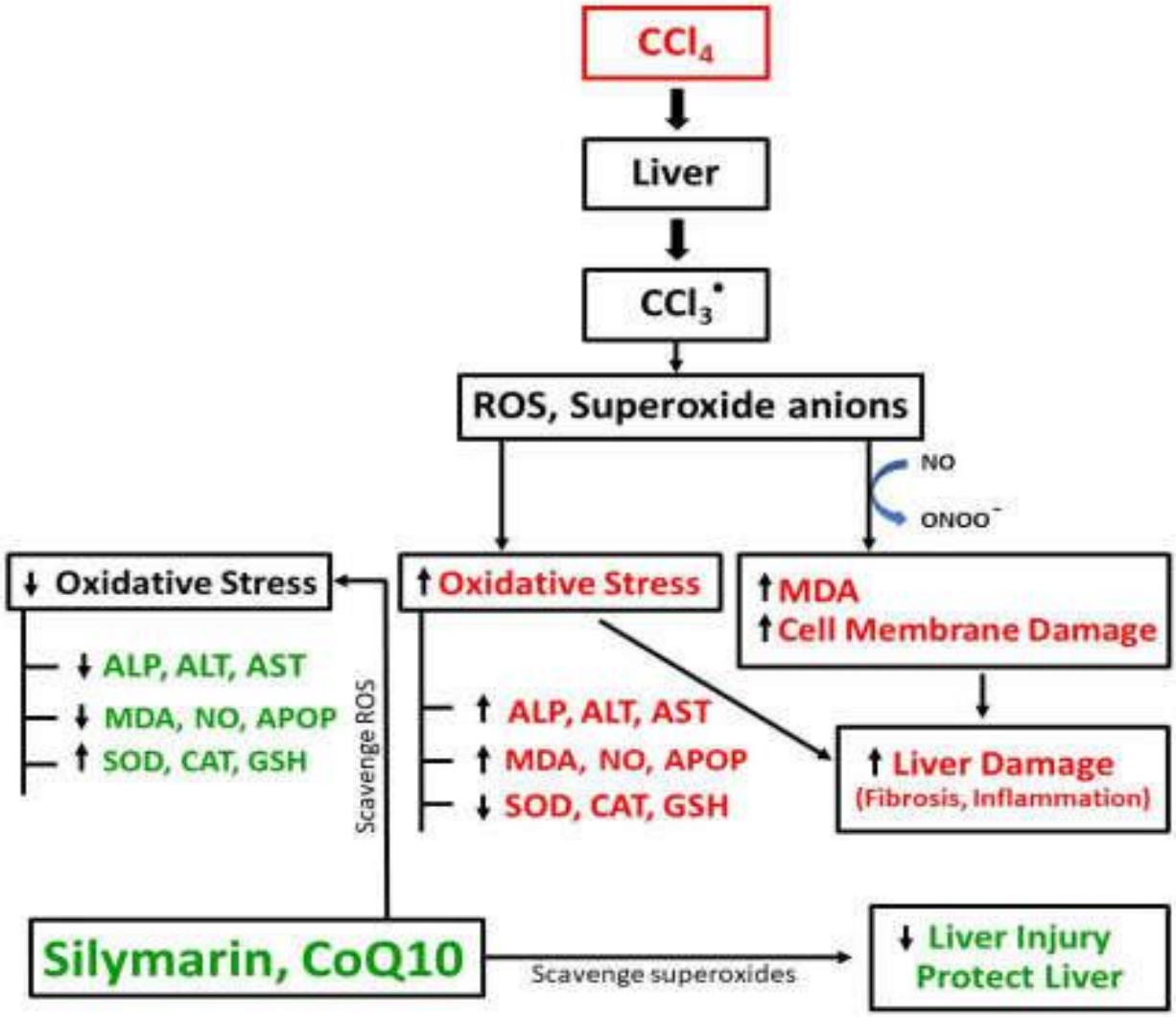


Fig 2.2.Mechanism of action of CCl4

Most cases of CCl4 poisoning result in damage to the liver, kidneys, and lungs. Through the use of experimental CCl4 poisoning, it is demonstrated that hepatocytes loaded with glycogen are far more resilient to damage than those with fatty infiltration and reduced glycogen.Chronic alcohol abusers are therefore more likely to experience more severe symptoms of CCl4-induced hepatotoxicity due to the depletion of glycogen and fat accumulation in their live cells. When CCl4 is inhaled, pulmonary damage is constant; however, when CCl4 is consumed orally,

pulmonary damage is negligible. (Washington *et al.*, 1958). CCl_4 toxicity may cause abnormal LFT parameters such as increased Aspartate Transaminase(AST), Alanine Transaminase (ALT), Glutamate Dehydrogenase (GDH), Total Protein, Bilirubin, Albumin and Globulin. These depends on the route and duration of toxic uptake. This toxin is absorbed rapidly in a great amount through inhalation than ingestion. So these parameters may increase within a very short period of inhalation is the route of toxin absorption.

2.4 The liver

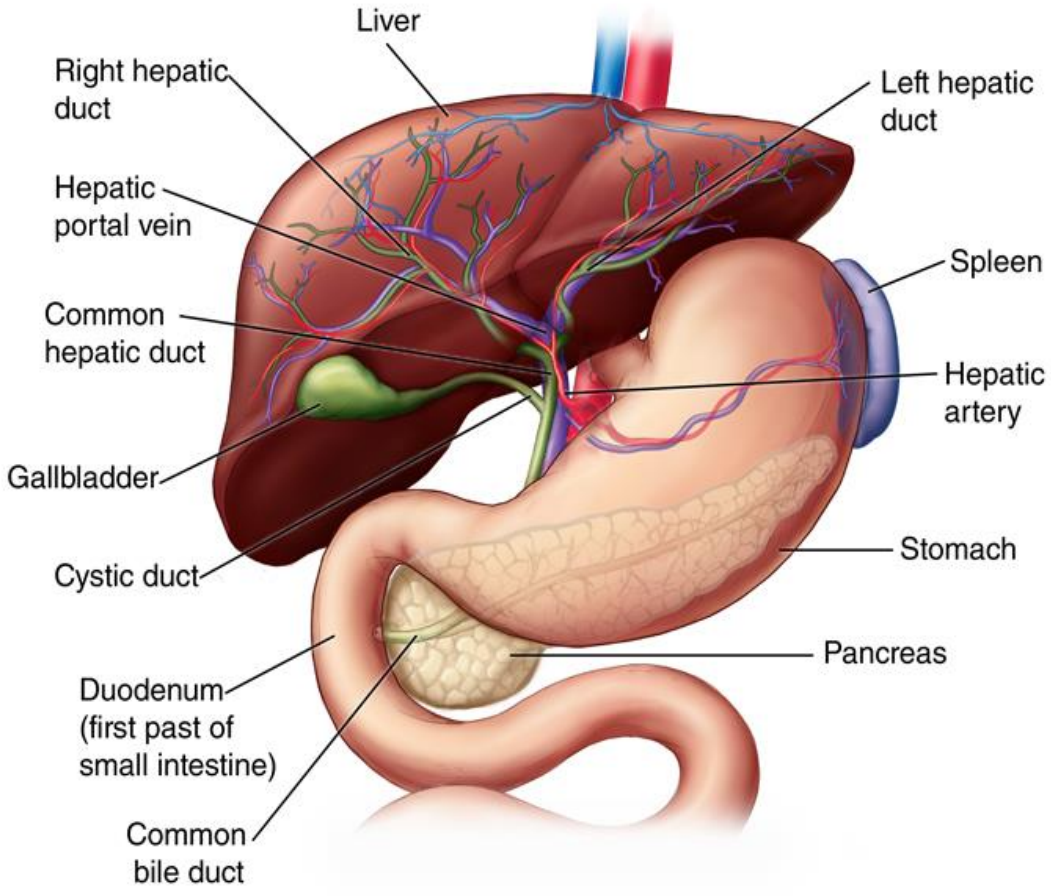


Fig 2.3.The liver

The liver is an essential organ involved in more than five hundred metabolic reactions in the biological system and one of its major functions is the detoxification of poison or harmful substances, but can also be injured by the toxicants which in the process may distort the metabolic activities of the liver, leading to acute liver failure .(Zamora-Nava *et al.;*2014).

The liver is prone to several pathologies, because of its incessant exposure to an environmental toxin, drug abuse, chronic alcohol intake, viral infections, and autoimmune diseases (Wolf *et al.*,2008) The treatment of liver diseases remains difficult despite the tremendous advancements in modern medicine. The current focus in the treatment of liver issues is on finding complementary and alternative medications with antioxidant qualities. Due to their advantageous effects on preventing disease and extending life, phytoconstituents—especially phenolic compounds—are becoming more and more popular.(Kris-Etherton *et al.*,2002).

2.5. Functions of liver

The major functions of the liver include:

Bile production: Bile aids in the breakdown and absorption of fats, cholesterol, and certain vitamins in the small intestine (Almajid et al., 2021). Water, electrolytes, cholesterol, bilirubin, and bile salts make up bile.

Absorbing and metabolizing bilirubin: Bilirubin is created when haemoglobin breaks down. Iron that is liberated from haemoglobin and used to create the subsequent generation of blood cells is stored in the bone marrow or liver.

Supporting blood clots: Vitamin K is necessary to create coagulants that help clot the blood. Bile is essential for vitamin K absorption(Vitamin k,2021) and forms in the liver. The liver must produce enough bile to make clotting factors.

Fat metabolization: Bile breaks down fats and makes them easier to digest.

Metabolizing carbohydrates: Carbohydrates are stored in the liver. Glycogen—stored carbohydrates in the liver—can be converted by the body into glucose. In order to control blood sugar levels and provide a brief energy boost, glucose, also known as sugar, is released into the bloodstream.

Vitamin and mineral storage: Vitamins A, D, E, K, and B12 are fat-soluble vitamins that are stored in the liver. It stores large quantities of these vitamins. When making new red blood cells, the liver stores iron from haemoglobin in the form of ferritin. Copper is also stored and released by the liver.

Helps metabolize proteins: Bile helps break down proteins for digestion.

Filters the blood: Compounds from outside the body, such as alcohol and other drugs, as well as hormones like oestrogen and aldosterone, are filtered and eliminated by the liver.

Immunological function: The mononuclear phagocytic system includes the liver. The liver contains large amounts of Kupffer cells, which are immune activity-related cells. Disease-causing viruses, bacteria, or other microorganisms that might enter the liver through the gut are eliminated by these cells (Almajid *et al*., 2021).

Production of albumin: The most prevalent protein in blood serum is albumin. It carries steroid hormones and fatty acids to help maintain proper blood vessel pressure and stop leakage.

Synthesis of angiotensinogen: This hormone causes the blood vessels to constrict when it detects the kidneys producing the renin enzyme, which in turn causes blood pressure to rise.

2.6. Diseases of the liver

The liver is a complex organ that can be affected by a variety of problems. When the liver is healthy, it functions optimally, but when it is diseased or not functioning properly, the consequences can be very serious, even fatal.

Examples of liver disease include:

Fascioliasis: Fascioliasis is a disease that can be brought on by a parasitic worm called a liver fluke invading the liver. Before showing any symptoms, the liver fluke can stay there for months or even years. (Fascioliasis, 2019).

Cirrhosis: This results in fibrosis, a process where liver cells are replaced by scar tissue. Hepatitis, alcohol, and toxins are just a few of the numerous things that can cause this illness. Liver failure may eventually result from fibrosis because it destroys the ability of liver cells to function.

Hepatitis: A general infection of the liver known as hepatitis can be brought on by viruses, toxins, or an autoimmune reaction. This disease is characterised by an inflamed liver. Although the liver can recover on its own in many situations, serious cases can result in liver failure.

Fatty liver disease: This usually happens in conjunction with alcohol abuse or obesity. Fat vacuoles accumulate in the liver cells of patients with fatty liver disease. When alcohol use is not the cause, nonalcoholic fatty liver disease develops.

Gilbert's syndrome: 4-16% of people have this genetic disorder. The body's breakdown of bilirubin is incomplete in Gilbert's syndrome. Although the disorder is harmless, mild jaundice may occur.

Liver cancer: Hepatocellular carcinoma and cholangiocarcinoma are the two most prevalent forms of liver cancer. Hepatitis and alcohol are the main causes. It is the third most common cause of cancer-related death worldwide and the sixth most common type of cancer overall.

2.7. Liver function test

Blood tests called liver function tests are used to monitor and diagnose diseases or damage that affect the liver. The tests quantify the concentrations of specific proteins, enzymes, and other substances produced by the liver in the blood. Tests for liver function include bilirubin, albumin, total protein, aspartate transaminase (AST), alanine phosphatase (ALP), and aspartate aminotransferase (ALT). A few of these tests gauge how well the liver is generating protein and eliminating bilirubin, a waste product of the bloodstream. Additional tests for liver function quantify the enzymes that the liver cells release in reaction to illnesses or damage.

2.8. Importance of LFTs

- Liver function test can be used for monitoring the progression of a disease, such as a viral or alcoholic hepatitis, and to determine how well the treatment is working.
- It is needed to measure the severity of a disease, particularly the scarring of the liver.
- It is also used to monitor the possible side effects of administered medications.
- It can be used to screen for liver infections, such as hepatitis.

2.9. Liver function test parameters

There are several types of Liver function test.

Alanine Transaminase (ALT): The liver contains an enzyme called ALT, which aids in the conversion of proteins into energy for the liver cells. ALT is released into the bloodstream and its level rises in response to liver damage.

Aspartate Transaminase (AST): AST is an enzyme that helps to metabolize amino acids. AST is present in the blood normally at low levels. An increase in AST levels may indicate liver damage, disease or muscle damage.

Alkaline phosphatase(ALT): This is an enzyme found in the liver and the bone and it is important for breaking down proteins. Higher than normal levels of ALP may indicate liver damage or diseases such as a blocked bile duct or certain bone diseases.

Gamma-glutamyltransferase (GGT): This is a blood enzyme that can cause liver or bile duct damage if levels are higher than normal.

L-Lactate dehydrogenase (LD): LD is an enzyme also found in the liver. An abnormal increase may be as a result of liver damage and also an elevated level of this enzyme can also signify other disorders.

Prothrombin time (PT): This is the duration it takes for the blood to clot. Increased levels of PT may Indicate liver damage, it can also be elevated if a certain drug thinning drug such as warfarin is being taken.

Globulins: They are proteins found in the blood. About 40% of the proteins in the blood are alpha, beta, and gamma globulins. Globulins help to form blood clots, fight off viruses and infections and keep the liver and kidney functioning properly.

Albumin and Total protein: The liver produces a variety of proteins, one of which is albumin. These proteins are necessary for the blood to fight infection and carry out other tasks. Decreases in albumin and total protein levels are indicative of diseases or damage to the liver. It is the primary component of total protein, with globulin making up the majority of the other components. Chronic liver disease, including liver cirrhosis, is associated with decreased albumin levels. Additionally, it is reduced in nephrotic syndrome, a condition in which the body excretes it in the urine. Since the intravascular oncotic pressure drops below the extravascular space, one of the effects of low albumin can be edema.(Smith,2017).

Bilirubin : One of the substances created during the regular lysis of red blood cells is bilirubin.It is a normal and healthy process for old cells to break down. Following blood circulation, bilirubin proceeds to the liver, where it is metabolised, combined with bile,

expelled via the bile ducts, and stored in the gallbladder.(Mayo clinic,2018). Bilirubin passes through the liver and is excreted in stool. Elevated levels of bilirubin (jaundice) might indicate liver damage or diseases or a certain type of anaemia. The liver sorts bilirubin with other waste products into a fluid called bile. High or low level of bilirubin might indicate that the liver is struggling in some way or the other in processing the breaking down and clearing of old red blood cells in the body. There are different types of bilirubin:

- **Indirect Bilirubin:** This is unconjugated bilirubin, which the liver has yet to process. Elevated levels can indicate excessive breakdown of red blood cells or issues with bilirubin uptake by the liver.

- **Direct Bilirubin:** This is conjugated bilirubin, which the liver has processed and is ready for excretion. Elevated levels suggest problems with bilirubin excretion.

Table 2.0.Reference range of liver function test parameters

Liver function test	**Normal Range**
Protein	6.6 - 8.3g/dl
Albumin	3.5 -5.2g/dl
Total bilirubin	0.2 - 1.3mg/dl
Alanine Transaminase	<40u/l
Aspartate Transaminase	<40u/l
Amylase	22 - 85u/l
Globulin	2.0 -3.5g/dl

CHAPTER THREE

MATERIALS AND METHODS

3.1.Apparatus

- Masking tape
- Cotton wool
- Filter paper
- Sieve Cloth
- Aluminum foil
- Normal latex gloves
- Measuring cylinder
- Conical flasks
- Buckets
- Tissue paper
- Stirring rods
- Weigh balance
- Nose masks
- Scissors
- Cages
- Feeding bowls
- Water trough
- Syringe
- *Chasmanthera dependens*
- Wistar rats
- Rats feed
- Plain bottles
- Funnels

- Pipette
- Spatula
- Handkerchiefs
- Binding wires
- Glues
- Centrifuge
- Lithium heparin bottles
- Cardboard papers
- Dissecting sets
- Beakers
- Disinfectants
- Cuvette
- Test tubes

3.1.1.Reagents used

- Ethanol
- Carbon tetrachloride
- Olive oil
- Distilled water
- Picric Acid

3.1.2. Equipment used

- Centrifuge
- Freeze dryer
- Incubator

3.2. Preparation of plants used.

The roots of *Chasmanthera dependens* were acquired from Oba Isin village, Kwara State. It was identified and authenticated by Dr. H.A Akinnibosun in the Department of Plant Biology and Biotechnology, University of Benin. The specimen samples were thereafter deposited at the department's herbarium with voucher number UBH_C387.

The plants were air dried under room temperature and not oven dried to avoid lost of important phytochemicals. After they were properly dried, they were pulverised and were weighed and soaked into a jar of hydroethanol with a ratio of ethanol to distilled water 70:30 respectively (700ml of ethanol and 300ml of distilled water). The mixture was stirred properly with a stirring rod and allowed to stand for 5days where it was stirred each day. After the fifth day, the extracts were filtered using a sieve cloth, basket sieves and lastly handkerchiefs and cotton wool was used for ultrafiltration. The extracts gotten was turned into a bottle and labelled according to it's contents. The crude extracts were taken to the Trigas lab to be freeze dried and kept in an air tight container afterwards.

3.3. Preparation of Animals Used.

25 male Wistar rats were purchased and housed in clean and well-equipped cages and were allowed to acclimatize for 2 weeks by consuming standard diet and water. The rats were divided into five groups with a maximum of 5 rats in a cage after acclimatization. The animals were weighed on a scale of weight to determine their various body weights, and each animal was labeled on various body parts such as their head, belly, back, left hand, and tail to indicate their respective body weight. These body parts of rats were stained with picric acid and gentian violet and were recorded for easy identification.

3.4. Experimental design

The wistar rats were arranged into five groups with the weight of the rats in a group being representatives of the weight range of all the rats.

Group 1: This is the normal control group. They were fed with normal feed and water and were not induced with CCl_4, but were administered Olive oil which serves as the vehicle and there was no form of treatment was administered.

Group 2: This is the negative control group. They were fed with normal feed and water. They were induced with CCL_4, but no treatment of any form was administered.

Group 3: This is the positive control group. They were induced with CCl_4 and treated with a known and standard drug called silymarin (140mg) for a duration of fourteen days.

Group 4: They were fed with normal feed and water. They were induced with CCL_4 and were treated with crude hydroethanol extracts of *Chasmanthera dependens* (250mg/kg) administered orally for a duration of fourteen days.

Group 5: They were fed with normal feed and water. They were induced with CCL_4 and treated with crude hydroethanol extracts of *Chasmanthera dependens (*500mg/kg) administered orally for a duration of fourteen days.

3.5. Inducing of liver damage with Carbon tetrachloride (CCl_4)

After the animals has acclimatised, administration of Carbon tetrachloride begins. CCl_4 was administered through intraperitoneal route using a syringe. It was prepared by diluting 5ml of

CCl_4 into 5ml of Olive oil. The animals were induced with CCL_4 twice and the Calculations and volumes are as below;

Dose of CCl_4 administered (ml)=(Body weight × 1.25)/1000

3.6. Preparation of the standard drug to be administered.

0.03mg of silymarin was added to a tablet of Silymarin and crushed to powder. It was dissolved in 7ml of distilled water and stirred until it was completely dissolved. This was done daily for 2weeks and the drugs were administered to the rats according to their weights.

3.7. Collection of Samples

Each rat was anesthetized with chloroform and was dissected. Blood was drawn from the heart region, through cardiac puncture and the sample was put in a lithium heparin bottle and centrifuged,and put in a plain bottle.

3.8.Procedure of liver function tests

3.8.1.Total protein

Serum protein concentration was measured by the biuret method (Gornall *et al*., 1949).

3.8.2 Principle

Biuret reagent which contains Cu^{2+} forms purple/blue complexes with peptide bonds under alkaline conditions which were read spectrophotometrically at 540nm.

3.8.3 Albumim

Serum albumin was measured by the Doumas and Biggs (1972) method, as described in the manual of the Randox albumin kit.

3.8.4 Principle

The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5, 5'-tetrabromo-m-cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

3.8.5 Procedure

To 0.01ml of plasma sample pipetted into a test tube, 3ml of BCG reagent R1, was pipetted into it. To another test tube, 0.01ml of standard solution and 3.00ml of BCG reagent was pipette into it and this serves as the standard. To prepare the blank, 0.01ml of distilled water and 3.00ml of BCG reagent was pipette into a different tube. The contents of each tube were separately mixed and incubated at 20-25^{0}C for 5minutes. The absorbance of the sample and standard tubes were measured against the reagent blank at 578 nanometer mercury using a 1cm light path cuvette.

3.8.6. Calculation

$$\text{Albumin concentration (g/l or g/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{Concentration of standard}$$

Where, A_{sample} = Absorbance of sample

$A_{standard}$ = Absorbance of standard

Concentration of standard = 46.4g/l, 4.64g/dl

3.8.7.Bilirubin

Serum bilirubin concentration was measured using Diazo reaction (Sykes and Epstein,1990) .

3.8.8. Principle

Total bilirubin is the presence of a solubilizing agent is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium. The intensity of the red azodye formed is directly proportional to the total bilirubin and can be determined spectrophotometrically (546nm).

3.9.Statistical analysis

Data was analysed using graph pad prism version 9.5 statistical software and the results analysed were presented in mean +/-SEM.

CHAPTER FOUR

RESULTS

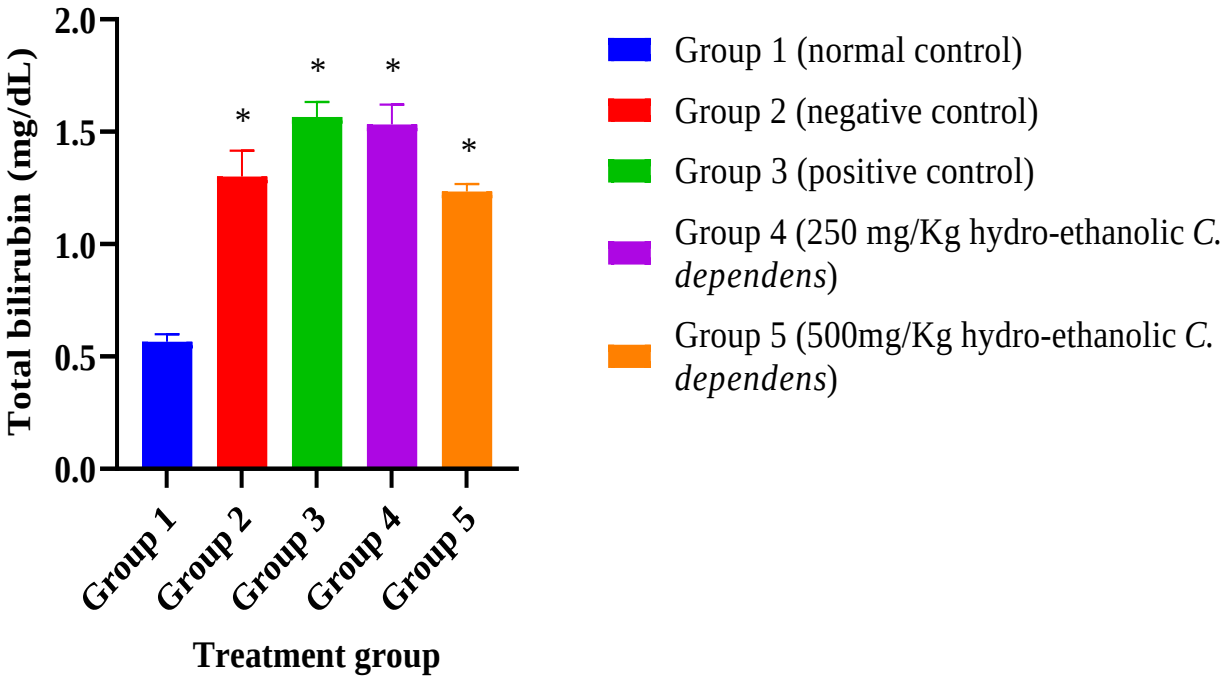


Figure 4.1: Effects of hydroethanol extracts of *Chasmanthera dependens* on total bilirubin in CCl_4 induced liver toxicity in male Wistar rats

* indicates a significant difference when the groups are compared to group 1 (normal control). Data are expressed as mean ± SEM (n =5). There was a significant difference in total bilirubin between the treatment groups, $p < 0.0001$ compared to normal control. Total bilirubin of rats in groups 2, 3, 4, and 5 was each significantly higher than that of group 1.

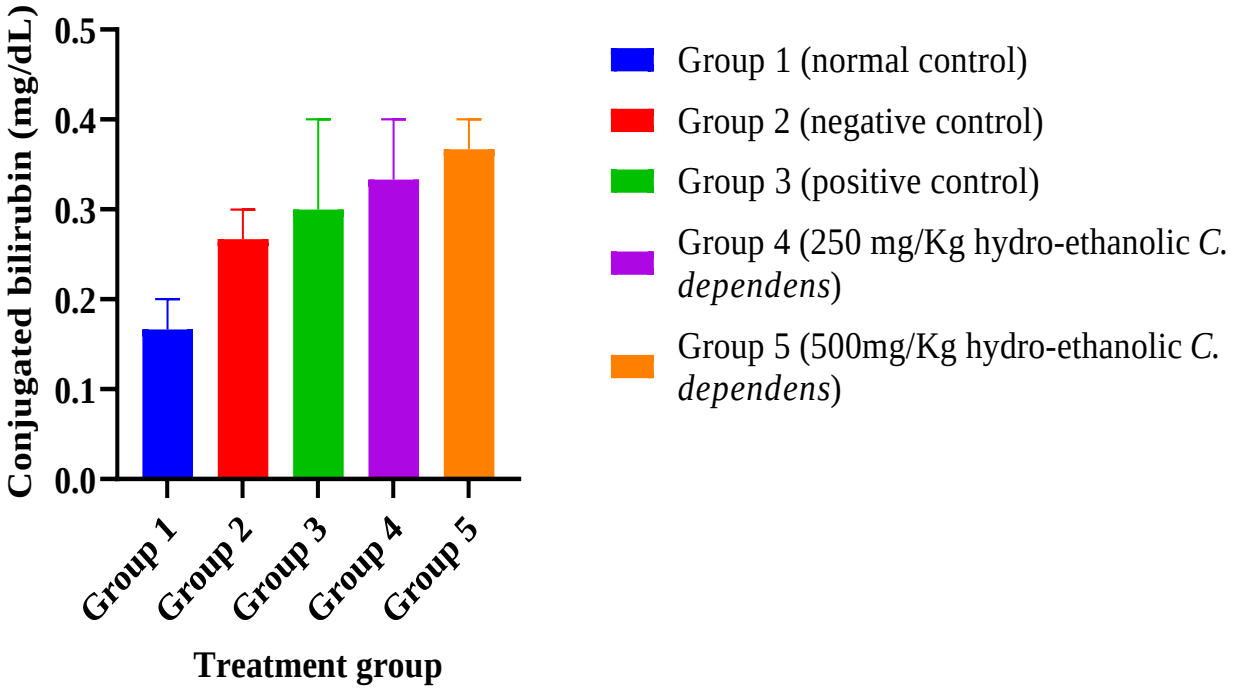


Figure 4.2: Effects of hydroethanol extracts of *Chasmanthera dependens* on conjugated bilirubin in CCl_4 induced liver toxicity in male Wistar rats

There was no significant difference in conjugated bilirubin between the treatment groups, p = 0.2356 compared to normal control.

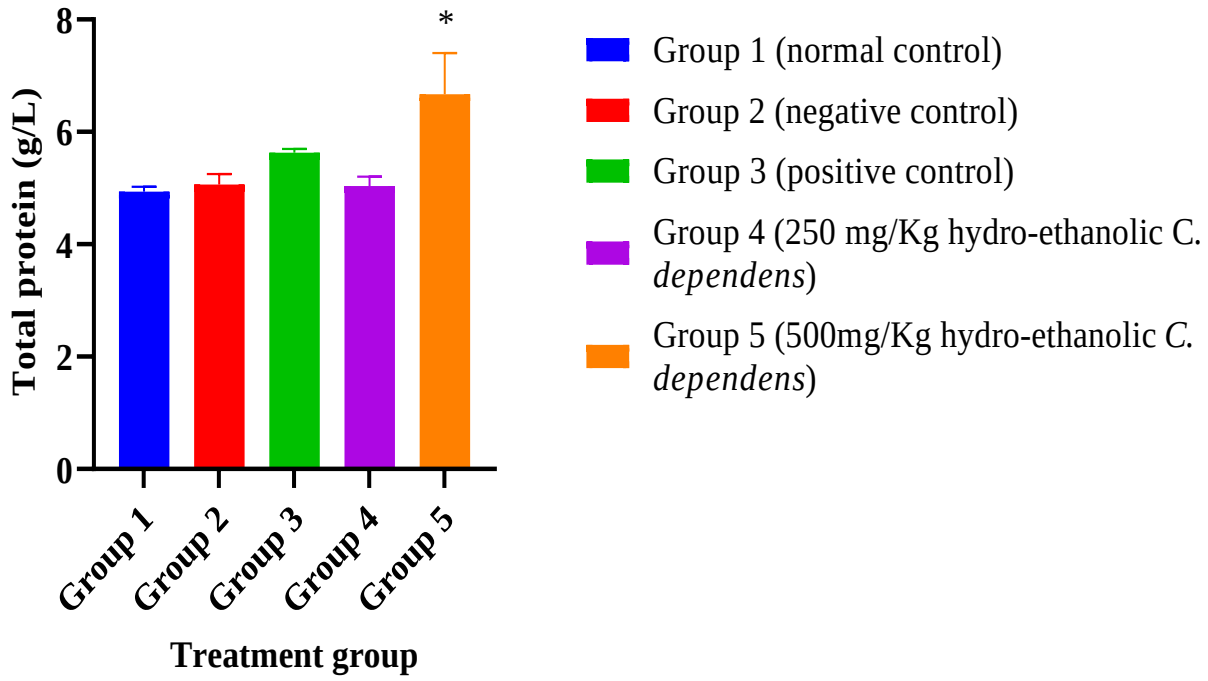


Figure 4.3: Effects of hydroethanol extracts of *Chasmanthera dependens* on total protein in CCl_4 induced liver toxicity in male Wistar rats

There was a significant difference in total protein between the treatment groups, p = 0.0282 compared to normal control. Total protein of rats in group 5 was significantly higher than that of group 1. Also, total protein of rats in group 5 was significantly higher than that of group 4.

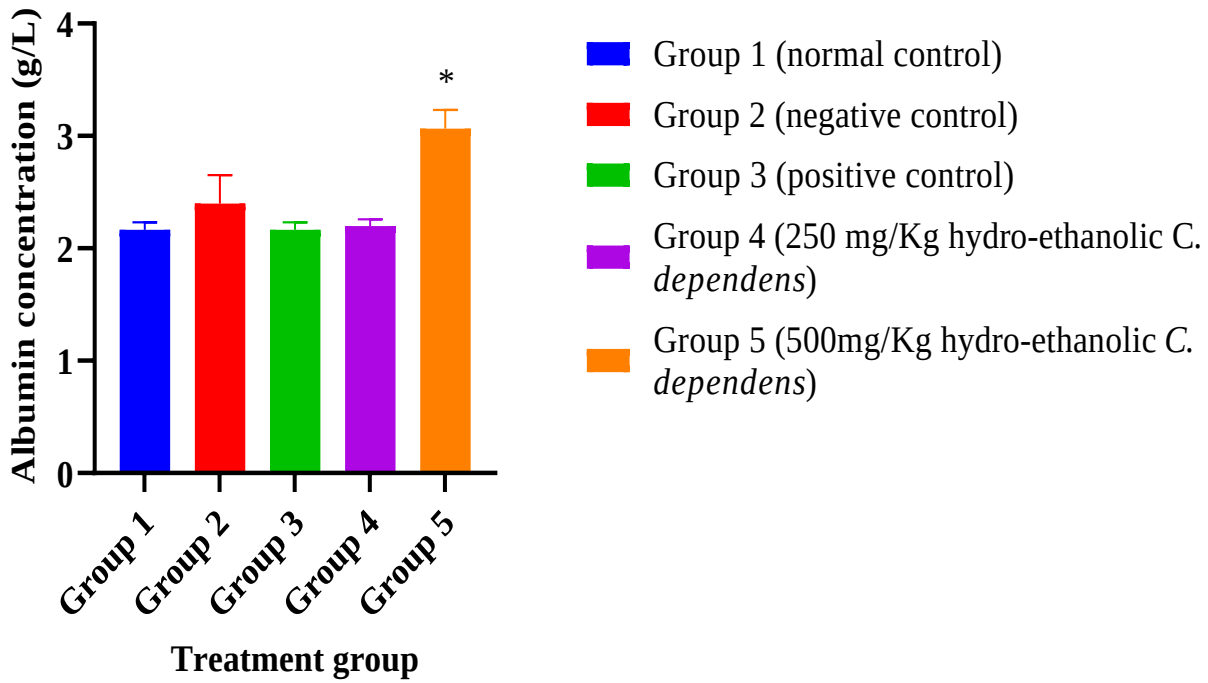


Figure 4.4: Effects of hydroethanol extracts of *Chasmanthera dependens* on albumin concentration in CCl_4 induced liver toxicity in male Wistar rats

There was a significant difference in albumin concentration between the treatment groups, p = 0.0054 compared to normal control. Albumin concentration of rats in groups 5 was significantly higher than that of group 1. Also, albumin concentration of rats in groups 5 was significantly higher than that of groups 3 and 4.

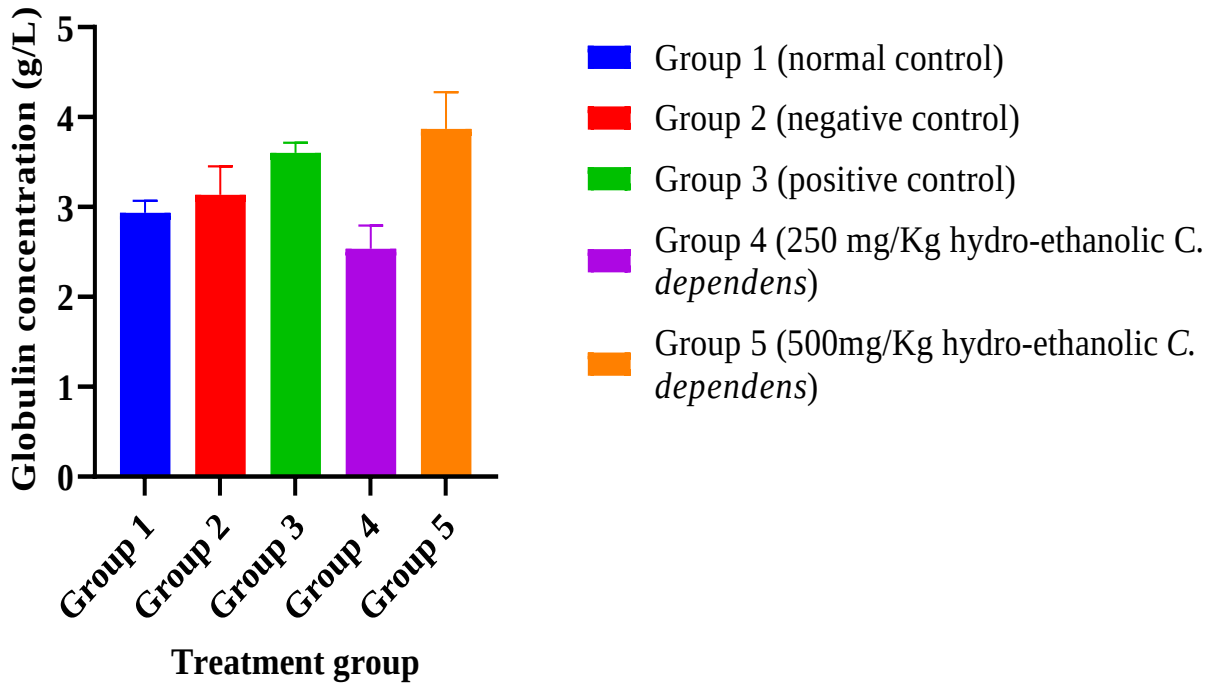


Figure 4.5: Effects of hydroethanol extracts of *Chasmanthera dependens* on Globulin concentration in CCl_4 induced liver toxicity in male Wister rats

There was a significant difference in globulin concentration between the treatment groups, p = 0.0390 compared to normal control. Globulin concentration of rats in groups 2, 3, 4, and 5 was not significantly different than that of group 1. However, globulin concentration of rats in group 5 was significantly higher than that of group 4.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. Discussion

The aim of this study was to investigate the potential hepato-protective effects of the hydroethanol extract of *Chasmanthera dependens* on liver function test parameters in a rat model of carbon tetrachloride (CCl_4)-induced hepatotoxicity. Liver diseases, especially those induced by toxins such as CCl_4, continue to be a significant global health concern.

The figures 4.1, 4.2, 4.3, 4.4, 4.5 displayed the effects of the extracts on the liver function test parameters on CCl_4 induces Wistar Rats. It was observed that the extracts had varying effects on each parameters when compared to the control group.

The results shows that there was a significant difference in total bilirubin at $p<0.0001$ among the treatment groups compared to normal control. The total bilirubin of rats in group 2,3,4 and 5 were significantly higher when compared to the normal control group. Conjugated bilirubin had no significant difference when compared to the normal control group.

There was a significant difference in total protein at $p= 0.0282$ compared to normal control between the treatment groups for total protein. It was observed that dose treatment at 500mg/kg significantly increased the concentration of total protein compared to the rats in group 2.

Likewise in the concentration of albumin, there was a significant difference at $p=0.0054$ when compared to rats in group 1 (normal control). It was also observed that group 5 which was treats with a dose of 500mg/kg body weight increased the concentration of albumin when compared to the rats in group 2.

There was no significant difference in globulin concentration of group 2,3,4 and 5 when compared to that of group 1.

These results which showed a significant increase in total bilirubin levels but no significant difference in conjugated bilirubin levels or globulin levels, suggest a specific pattern of liver dysfunction in the context of carbon tetrachloride (CCl_4)-induced toxicity and treatment with the hydroethanol extract of *Chasmanthera dependens*. CCl_4 causes cellular damage in multiple organs, mostly in the liver, kidney and lungs. (Teschke ,2018), (Slater *et al*.,1985).

The significant increase in total bilirubin levels indicates that there is an overall rise in bilirubin in the bloodstream. Elevated total bilirubin levels can be indicative of impaired liver function or an obstruction in the bileducts. Futhermore, the lack of significant difference in conjugated bilirubin levels might be as a result of an elevation in unconjugated (indirect) bilirubin. This lack of change in conjugated bilirubin levels could imply that the liver's ability to conjugate bilirubin remains relatively intact.

The absence of a significant difference in globulin levels indicates that *Chasmanthera dependens* treatment did not have a significant impact on the synthesis of globulins, This suggests that the treatment did not significantly influence the overall protein profile.

The observation that Group 5 (receiving 500mg/kg hydro-ethanolic *Chasmanthera dependens*) showed a significant increase in total protein and albumin compared to the other groups suggests a positive effect of this treatment on liver function parameters, specifically in terms of protein synthesis and maintenance. A significant increase in total protein and albumin levels in Group 5

implies that the liver's ability to produce proteins may have been enhanced by the higher dose of *Chasmanthera dependens* .

This results contrast with Quadri and Yakubu, 2015 whose dose treatment at 25mg/kg, 50mg/kg and 100mg/kg of aqueous *Chasmanthera dependens* led to a decrease of total protein and albumin concentration, an increase in globulin concentration and and increase in total bilirubin and conjugated bilirubin.

5.2. Conclusion

This outcome indicates that the 500mg/kg dose of hydro-ethanolic *Chasmanthera dependens* had a more pronounced positive impact on liver function, particularly in terms of protein synthesis and maintaining blood protein levels, compared to the other doses and control groups. It suggests that this higher dose may hold promise for potential therapeutic use in supporting liver health and function. Further research may be needed to understand the specific mechanisms underlying this effect.

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