NUTRITIONAL COMPOSITION, CHARACTERIZATION OF SOME PHYTOCHEMICAL CONSTITUENTS AND HEPATOPROTECTIVE ACTIVITY OF POLY-HERBAL TEA FORMULATION (MORINGA OLIFERA, TURMERIC, GINGER, GARLIC AND LEMON) IN CCL4-INDUCED HEPATOTOXICITY.

BY

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UNIVERSITY OF BENIN

BENIN CITY.

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT FOR THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY.

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CERTIFICATION

This certify that this seminar work was carried out by **Victory Amenaghawon EGHAREVBA (Miss)** with matriculation number **LSC1605935** under the under the supervision of **Dr. D. O. Uwaya** Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

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DEDICATION

This project work is dedicated to God Almighty who is my source of wisdom and knowledge and I also wish to dedicate it to my parents and siblings who deemed it fit to provide the encouragement and support for the success of my project work.

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My sincere gratitude goes firstly to God Almighty for his grace, provision, strength and peace throughout this project, may His name be praised forever (Amen).

It was indeed an uphill task to have been able to put together this review work and it is on this note that I specially thank my supervisor **Dr. D. O. Uwaya** for his supervisory support, effort and understanding throughout this work. I say thank you sir and may God reword you effortlessly.

Let me also express my gratitude to the head of department **Associate. Prof. E. O. Oshomoh** and to my loving parent **Mr. & Mrs. V.U Egharevba** and my wonderful husband **Engr. Kingsley O. Ehigiamusor (MNSE, COREN)**.

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LIST OF ABREVIATIONS

CCL4- Carbon tetrachloride

HPLC- High performance liquid chromatography

ALP-Alkaline phosphate

AST-Aspatate amino transeferase

ALT-Alanin Amine Transaminase

SOD-SuperoxidDismutase

MDA-Molondialdehyde

ABSTRACT

Polyherbal teas, also known as herb-herb combinations, have been used in Chinese medicine practice, but scientific evidence of their therapeutic benefit is lacking. This study aims to examine the nutritional composition, characterization of some phytochemical constituents, and hepatoprotective activity of a poly-herbal tea formulation comprised of *Moringa olifera*, turmeric, ginger, garlic, and lemon in CCl_4-induced hepatotoxicity. Proximate and mineral compositions were analyzed by the method described by the Association of Official Analytical Chemists (AOAC). The characterization of some phytochemical constituents was analyzed using HPLC. CCL4 induced hepatotoxicity was used for hepatoprotective activity. The proximate composition of poly-herbal formulated tea indicated carbohydrate (64.66 %), protein (19.25 %), fat (6.35 %), moisture content (6.12 %), ash content (0.24 %) and fibre (3.36%). Mineral compositions present include potassium (1356.0 mg/kg), calcium (821.3 mg/kg), magnesium (380.8 mg/kg), phosphorus (331.4 mg/kg), and iron (221.4 mg/kg).nt). Luteolin, Arbutin, Kaempferol, Apigenin and Quercetin were the most abundant phenolic compound, Quinine was the most abundant alkaloid, Diosgenin and Ergocalciferol were the most abundant steroid, Epigattotechin and Catechin were the most abundant tannins in polyherbal formulated tea. The body weight of animals given 10 mg/kg of the formulated tea extract, 5 mg/kg of the tea extract + CCL4, 10 mg/kg of tea extract + CCL4 and CCL4 without treatment significantly reduced compare to control (*$p<0.05$) in CCL4-induced nephrotoxicity. There was liver weight reduction in the animals that were given distilled water, 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 when compared with CCL4 without treatment (***$p<0.001$; *$p<0.05$). ALP, AST and ALT levels in the animals were reduced by 5 mg/kg of the formulated tea extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$). Superoxide dismutase and catalase values were increased, and the molondialdehyde level was reduced by the formulated

tea extract when compared with CCL4 without treatment (****p<0.0001; ***p<0.001; **p<0.01; *p<0.05). Conclusively polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric and lemon) possesses hepatoprotective activity.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Throughout history, eighty to eighty-five percent of the world's population has relied on traditional medicines for health care (Prakish et al., 2013). Natural goods have been made from plants for thousands of years, and more recently, their potential medical advantages inside the treatment of various illnesses have been examined because of their capacity to mitigate the negative impacts of oxidants on diets (Hirasa and Takemasa, 1998; Panovska *et al*., 2005; Carović-Stanko *et al*., 2016). The usage of medicinal plants for the managing of human illness has been linked to the phytochemical compound present in them, which gives exact physiological actions both in humans as well as in animals (Sen *et al*., 2010). Medicinal plants have some exceptional and diverse characteristics compared to canonical drugs in that they are accessible, non-expensive, long-lasting, and have a wider range of therapeutic uses when compared to artificial drugs (Calixto, 2000; Idu, 2010). Plants contain a amount of unique organic phytochemicals (including alkaloids, flavonoids, phenols, saponins, terpenoids, etc.) that have been linked to good health (Mihaylova *et al*., 2018). According to Lillehoj and Lee (2012), phytochemicals are indeed suggested for usage as antioxidants into animal feed to help animals from the oxidative damage produced by free radicals. They are also thought to have the ability to enhance effects and/or mitigate adverse effects (Gilani and Atta, 2005). The biological actions of plant bioactive compounds, commonly referred to as phytochemicals, include antibacterial, anti-inflammatory, anti-helmintic, analgesic, neuroprotective, as well as immune-modulatory impacts on both human coupled with animal health (Dhan et al., 2012). Other components found in plants having therapeutic qualities include tannins, alkaloids, glycosides,

and phenols. In addition to geographical area, type of soil, plant age, as well as harvesting and extracting techniques, these components in plant extracts differ through one plant to another (Hyun et al., 2016).

In Chinese medicine, polyherbal treatments, sometimes known as herb-herb combos, were utilized for thousands of years, although there is little scientific proof of its therapeutic value (Che et al., 2013). It has been discovered that naturally occurring herbs as well as herbal compounds included in certain formulae may interact to produce effects including mutual augmentation, mutual aid, mutual restraint, as well as mutual antagonism (Ramaish *et al*., 2013). The Ayurvedic medical system focuses primarily on the use of polyherbal substances in order to cure a wide variety of infectious diseases. Examples of polyherbal compounds widely used in Ayurvedic medicine for various ailments are Bharangyadt (a mixture of *Clerrodendrum serratum, Hedychum spicatum, and Inula racemosa),* and Indukanth Ghiritha (IG), a polyherbal preparation consisting of seventeen plant components (George *et al*., 2008). Some plants mentioned in Ayurveda are highly known for their medicinal properties. Among these are *Moringa olifera*, garlic, ginger, turmeric, and lemon.

1.2 *Curcuma longa* (Tumeric)

1.2.1 Description

The rhizomatous perennial plant turmeric (curcuma longa), a member of the Zingiberaceae family, is frequently utilized as a culinary spice, a body cleansing, and as medicine to cure a variety of conditions, including anorexia, coughing, sinusitis, asthma, renal and hepatic ailments. (Choi *et al*., 2019). It may reach a height of one meter and has a stem that is just a few

centimetres long. Its leaves are oblong and pointed, and its blooms are funnel-shaped and yellow.

1.2.2 Distribution

The majority of the world's tropics in addition subtropical climates were home to it, although it is mostly farmed in Asian nations, most notably India and China. Its natural range encompasses almost the whole planet. "Haldi," as its a traditionally recognized inside India, has rhizomes that were oblong, ovate, pyrifomed, besides frequently branched (Jaggi, 2012; Roshan, 2017; Akram, 2010).

1.2.3 Ethnomedicinal uses

Current research has shown which *curcumin* has a new magnitude to their potential as well as has anti-inflammatory as well as anticancer activities (Naga, 2016). Curcumin is a yellow powder that may be produced from the rhizome of the turmeric plant as well as utilized in therapeutic applications. The yellow hue of curry powder comes from the dried Curcuma longa plant, which is also the origin of the spice known as turmeric. In addition to its application in ancient Indian medicine as well as Hindu religious rituals, turmeric are also extensively utilized in the culinary industry for both the flavour as well as colour it imparts to meals. Turmeric is a carminative and an aromatic stimulant, according to ancient Hindu literature (Roshan, 2017; Akram, 2010). Powdered turmeric has been utilized as a conventional treatment for gastrointestinal ailments, particularly biliary as well as hepatic problems, wound from a diabetic patient, rheumatism, inflammatory processes, sinusitis, anorexia, coryza, as well as cough, among other conditions, relatively recently. Turmeric, that plays as anticancer, antioxidant, anti-

inflammatory, anti-fertility, hepatoprotective, anticoagulant, as well as possess anti HIV effects towards fight AIDS (Akram, 2010; Naga, 2016; Pandey, 2013).

1.3 Moringa olifera

1.3.1 Description

Moringa olifera is a drought resistant herb from the family Moringaceae (Kou *et al*., 2018). It should come as no surprise that *M. oleifera* may be referred to as "Mother's Best Friend," "Miracle Tree," "Tree of Life," as well as "God's Gift to Man" (Johnson, 2005; Mbikay, 2012).

1.3.2. Distribution

Numerous tropical and subtropical nations in Asia, Africa, and other parts of the world grow the plant *Moringa oleifera* (MO) (Ajayi et al., 2016). Although in the Nile Valley, it also is referred to as "Shagara al Rauwaq," meaning means "tree for purifying," it is most often called the drumstick tree otherwise horseradish tree (Mbikay, 2012). (Von Maydell, 1986). It is utilized as a leafy vegetable having leaves that may be consumed raw, cooked, or dried and kept for several months without refrigeration otherwise nutritional value loss. (Anwar *et al*., 2007).

1.3.3. Ethnomedicinal use

It has traditionally been utilized to treat a wide range of illnesses, such as hyperglycemia, lithiasis, hypertension, hepatotoxicity, inflammation, fever, as well as asthma (Khan et al., 2017; Ingal and Gandhi, 2016). Moringa oleifera leaves' According to Aslam et al. (2005) and Gowrishankar et al. (2010), the plant's trace metal ions, especially potassium, phosphorous, manganese, and iron, along with vitamins A, D, E, and C, as well as alkaloids like moringi-9 and carotenoids like -carotene, are all believed to work together to produce therapeutic benefits. Additionally, M. oleifera has three structural groups of phytochemicals, each of which has a variety of therapeutic advantages. They consist of flavonoids like quercetin along with kaempferol, glucosinolates like glucomoringin, phenolic acids like chlorogenic acid, and

flavonoids like quercetin (Mbikay, 2012). Several phytochemicals are thought to offer anti-inflammatory, antioxidant, diabetic, hypotensive, and anti-cancer activities (Lako *et al*., 2007; Manguro and Lemmen, 2007; Amaglo *et al*., 2010; Kasolo *et al*., 2010).

1.4 Zingiber officinale Rosc

1.4.1 Description

Zingiber officinale Rosc., this as a herbaceous plant containing fragrant and spicy rhizomes that is endemic to northeast India is called simply as "ginger." (Baliga *et al*., 2011). Herb without stems that has a root stock The leaves may be widely lanceolate or oblong in shape, coupled with they have a dark ferruginous purple colour. The sheath as well as the petiole are both the same length as the blade. The spikes come out first, followed by the leaves. Flowering bracts are green with a ferruginous tinge; flowers are pale yellow, turning reddish at the outer border. (Sayantani and Ramachandra, 2019)

1.4.2 Distribution

It is a crucial component of conventional herbal medicine in China (Ali et al., 2008). In addition, ginger is regarded as one of the standard seasonings in global food in general and Moroccan cuisine throughout particular (Alami *et al*., 2015). Geographically, ginger is extensively grown in around 50 nations, majority of which are found within the tropics as well as subtropical zones. India, China, as well as Nigeria seem to be the top three producers, accounting for 35, 19, coupled with 12 percent of global output, correspondingly. With an annual capacity for production of over 1.07 million tons during 2017, India was without a doubt the world's greatest producer of ginger. The states of Kerala, Karnataka, as well as Northeast India generate the majority of the country's ginger. In addition to being provided towards the Indian market, it is

additionally exported, at such an approximate 29.6 percent, toward Morocco, the United States, Bangladesh, and the United Kingdom. (Zhang *et al*., 2020).

1.4.3 Ethnomedicinal uses

There are several pharmacological properties of this herb, including hypolipidemic actions (Alami *et al*., 2015; Irannejad *et al*., 2020; Beckkouch *et al*., 2019); antioxidant activity; an antidiabetic effect (Beckkouch *et al*., 2019; Danciu *et al*., 2015; Taha *et al*., 2014; Kim *et al*., 2018); coupled with anticancer activity (Eikirdasy *et al*., 2015).

1.5 Citrus limon

1.5.1 Description

A lemon, also known by its scientific name *Citrus limon L*., is a member of the Rutaceae family of fruits. (Goetz, 2014).

1.5.2 Distribution

This is a shrub having Southeast Asian roots that is grown around the globe throughout semi-tropical climates, including on the Mediterranean coast (Debuigne and Couplan, 2008).

1.5.3 **Ethnomedicinal** uses

Lemon is well recognized for its various healthful physiological benefits, including its anti-inflammatory properties (Tag *et al*., 2014; Haidari *et al*., 2019), lipid-lowering activity (Lee *et al*., 2018), antioxidative activity (Aazza *et al*., 2011; Klimek *et al*., 2020), coupled with anticancer and antimicrobial properties (Klimek *et al*., 2020).

1.6 Garlic (Allium sativum)

1.6.1 Description

Garlic (*Allium sativum),* commonly known as clove garlic, is a member of the family of the Lilliceae as well as as being often used as a flavour in cooking. However, during both ancient and contemporary history, it has also been utilized as a medicine for the treatment and prevention of illnesses and disorders (Zubair et al., 2019). It is a perennial bulbous plant associated with onions. This has a blooming stem which is 2 to 3 feet tall as well as upright. The plant produces pink otherwise purple blooms within middle towards end of the summer.

1.6.2 Distribution

Although it is a perennial bulb that is native throughout Central Asia, Siberia, as well as the western Himalayas, garlic were cultivated within England since around 1540. Garlic is native from Central Asia as well as the western Himalayas.. Today, it is extensively grown around the globe (Zubair et al., 2019) The place of Allium sativum is rather straightforward. It is often cultivated in a region with mild winters as well as warm summers, somewhat unlike to that of Central Asia. It grows in both the northern as well as southern hemispheres, but only by farmers (Zubair et al., 2019).

1.6.3 Ethnomedicinal use

According to the medical text Codex Ebers (circa 1550 BC), garlic played a significant role throughout ancient Egyptian medicine, particularly for the middle class engaged in hard work. This was due to garlic had been an efficient treatment for a variety of ailments, including heart issues, headaches, bites, worms, as well as tumors (Thomson, 2007; Zubair *et al*., 2019). S-allyl cystein sulfoxide, also known as SACS, is an amino acid that contains sulphur. The bulk of SACS's therapeutic capabilities are thought to be caused by this sulphur such as their ability to control lipid peroxidation more effectively than glibenclamide or insulin. SACS is the precursor of allicin as well as garlic oil.

1.7 AIM OF THE STUDY

The aim of this study is to evaluate the nutritional composition, characterization of some phytochemical constituents, and hepatoprotective activity of poly-herbal tea (moringa olifera, turmeric, ginger, garlic, and lemon) in CCl_4-induced hepatotoxicity.

1.8 SPECIFIC OBJECTIVES OF THE STUDY

I. To determine the nutritional composition present in poly-herbal tea (Moringa olifera, turmeric, ginger, garlic, and lemon),
II. To characterize the phytochemical constituents present in poly-herbal tea (moringa olifera, turmeric, ginger, garlic, and lemon).
III. To determine the hepatoprotective activity of a poly-herbal tea (Moringa olifera, turmeric, ginger, garlic, and lemon) in CCl_4-induced hepatotoxicity

CHAPTER TWO

LITERATURE REVIEW

2.1 LIVER AND ITS FUNCTIONS

Its liver, the biggest gland inside the body, makes a considerable contribution towards the coordination as well as maintainence of crucial physiological processes, that in turn helps to maintain homeostasis (Haque *et al*. 2017; Khan *et al*. 2020). It is engaged in the disintegration as well as removal of poisons like medicines as well as other foreign chemical compounds as

well as the evacuation of waste metabolites. Nutrients like as lipids, proteins, as well as carbohydrates are also broken down as well as eliminated via this process. A liver is a huge gland which carries out a variety of tasks, mostly those related to metabolism but also those related to biliary secretion, as well as excretion (Bates and Bickley, 2014). Nevertheless, various toxic exterior factors (drugs, commercial chemicals, heavy metals, etc.) that may result in cirrhosis, steatosis, as well as necrosis—all of which are together referred to as "hepatotoxicity"—threaten its very existence. (Loichot *et al*., 2004). Liver illnesses affect and over 10percent of a worldwide people, and their final stages are often followed by liver tumors and cirrhosis (Murriel et al., 2017). In various disease situations, persistent acute otherwise chronic exposure towards hepatotoxic substances often causes an excessive formation of free radicals, including reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Chattarjee et al., 2000). Those free radicals could change any kind of molecule otherwise cell, perhaps doing irreparable structural otherwise functional harm over the long run (Liu et al., 2009). Nevertheless, liver toxicity along with drug-induced oxidative stress are the chief reason of hepatic dysfunction (Abbound et al., 2007). A number of laboratory animals are regularly treated with the well-established hepatotoxic chemical carbon tetrachloride (CCL4) towards treat both acute and chronic liver injury (Abbound *et al*., 2007; Ma *et al*., 2014; Kaneko *et al*., 2013). That's because the liver is a key metabolic junction inside the body's operation, keeping the liver in excellent health is vital aimed at preserving the homeostasis of the body's. Over the years, there has not been any effective treatment inside conventional otherwise artificial medicine for protecting the liver in contrast to damage (Nithianantham *et al*., 2011). Respectively, For such treatment of liver illness, attempts are being undertaken to locate potential curative agents within natural products with lesser adverse reactions.

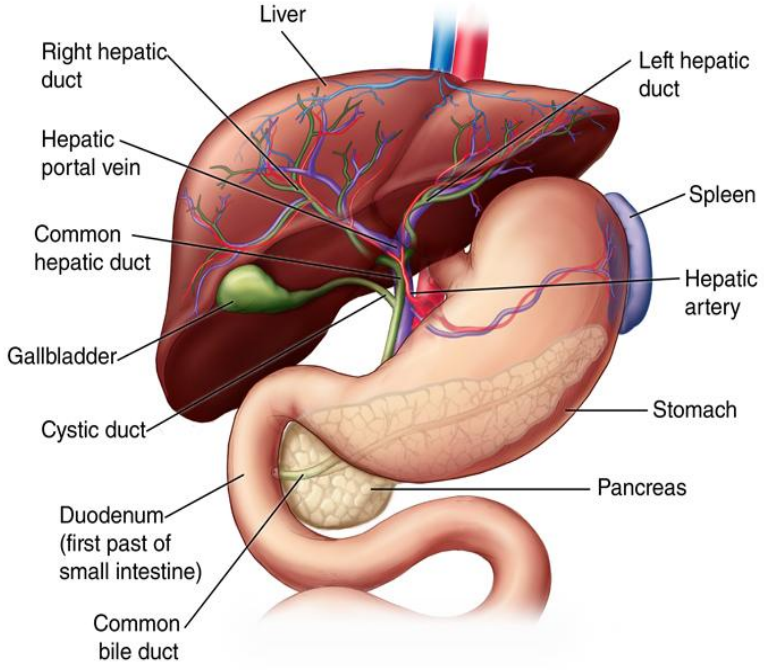


Figure 1: Anatomy of the liver

2.2 HEPATOTOXICITY AND LIVER DISEASES

Liver illness is the leading cause of death on such an annual basis. According to Lachier et al. (2013), and over 30 million Americans possess liver illnesses, and around 29 million individuals get a chronic liver problem (American Liver Foundation, 2017). Among the major worldwide health problems affecting poor nations is liver disease. Hepatiosis (non-

inflammatory), acute or chronic hepatitis (inflammation), as well as cirrhosis or fibrosis are the distinct classifications for these illnesses (degenerative). They are often brought on by heavy metals, pollutants, starvation, coupled with unauthorized use of over-the-counter medications. The preceding causes ultimately lead to hepatitis, jaundice, coupled with alcoholic liver problems by destroying coupled with incapacitating the hepatocytes. Since the liver has been the primary organ coping handle oxidative stress, persistent liver failure might well have major health effects including fibrosis, that can result in cirrhosis as well as liver cancer (Mohamed et al., 2016). An rise in blood cholesterol levels can be one of the symptoms of liver disease or injury. A significant risk of cardiovascular illnesses is associated with high levels of triacylglycerols (TAGs) and low-density lipoprotein cholesterol (LDL-C). (Okaiyeto et al., 2018; Dominiczak, 2005). Additionally, too much alcohol intake, chemical toxins like thioacetamide (TAA), the misuse of specific drugs like paracetamol (PCM), chemotherapy drugs like carbon tetrachloride (CC14), as well as various organic and inorganic compounds, microbes, and viral diseases (for instance, hepatitis A, B, C, and D), have all been carefully examined as causes of hepatic cell injury. (Ajboye et al., 2010; Sathesh et al., 2009) Reactive oxygen species (ROS, CCl3O), that are produced as a consequence of the endoplasmic reticulum and mitochondrial cytochrome P450's metabolism of CC14, start a chain reaction that may or may not cause lipid peroxidation. The liver cirrhosis with humans is identical towards the damage reactions brought from the infusion of carbon tetrachloride in a rat model (Weiler-Normann et al., 2007). One of several processes that causes oxidative damage towards liver cells would be the production of free radicals by CCl4 (Alam et al., 2018). Chronic hepatitis C virus disease, excessive drinking, and non-alcoholic steatohepatitis seem to be the biggest factors of liver fibrosis (Gine`s *et al*., 2004).

2.2.1 .Acute Liver Failure (Julie et al., 2005)

The liver experiences acute liver failure when it appears to have lost its capacity to operate. Frequently, liver failure develops slowly over many years. After several days, acute liver failure manifests. Numerous problems, such as severe bleeding as well as raised blood pressure inside the brain, may result from acute liver disease. Acute liver failure is also known as fulminant hepatic failure. Acute liver failure necessitates hospitalization as a medical crisis. Therapy may be able to reverse certain acute liver disease causes. However, in certain cases, the only treatment for acute liver disease may need a liver transplant.

2.2.2. Hepatitis (Dienstag, 2008)

Hepatitis is characterized by liver swelling coupled with inflammation. The phrase is frequently employed to describe a viral liver illness. Diseases involving viruses (including Hepatitis A, B, C, D, as well as E), bacteria, otherwise parasites, liver problems through alcohol, toxic mushrooms, or other toxins, drugs including an excess of acetaminophen, which may be fatal, or hepatitis can all be causes of the disease. Additionally, hereditary diseases like hemochromatosis as well as cystic fibrosis, which entail possessing much more iron within the body, may result in liver illness (the liver's high iron accumulation). Wilson's illness is one of the other reasons (excessive body copper deposits). Hepatitis may cause stomach discomfort or distention, male breast growth, dark coloration urine, pale or clay-colored feces, weariness, generalized itching, jaundice (yellowing of the complexion as well as eyes), lack of appetite, nausea, vomiting, as well as losing weight. These are only a few of the symptoms.

A. Hepatitis A

The hepatitis A virus causes the liver to become inflamed (irritated as well as swollen). Affected individuals often have the hepatitis A virus in their blood as well as feces for 15 to 45 days before to the onset of symptoms as well as the first week after becoming unwell. Typically, 2–6 weeks after hepatitis A virus exposure, symptoms start to manifest. Even though they are often moderate, particularly in adults, they could linger for up to many months. Dark urine, itching, lack of appetite, nausea, anorexia, malaise, pale or clay-colored stools, as well as yellow skin (Koff and Elisabetta, 1998; Meleleo *et al.*, 2012).

B. Hepatitis B

Hepatitis B is a illness further with hepatitis B virus which causes inflammation as well as irritation of a liver (HBV). By coming into touch with the serum or bodily fluids (including saliva, vaginal secretions, or semen) of a carrier, hepatitis B may be transmitted. Hepatitis B manifestations can take as long as six months to manifest after the infection. Early symptoms such as lack of appetite, fatigue, muscle as well as joint aches, nausea coupled with vomiting, dark urine coupled with hepatomegaly (Lee, 1997).

C. Hepatitis C

A viral condition called hepatitis C causes the liver to enlarge (inflammation). The hepatitis C virus is what causes hepatitis C infection (HCV). Interaction with the serum of a person who carries the virus causes the transmission of hepatitis C. Hepatitis C infections may cause considerable symptoms: Right upper abdominal discomfort, fluid-related abdominal swelling (ascites), clay-colored or light-colored feces, dark urine, exhaustion, fever, itchiness, jaundice, absence of appetite, coupled with vomiting are some of the symptoms that might occur.

D. Delta Agent (Hepatitis D)

Hepatitis D is a subtype of the virus known as delta agent. Only those that have a hepatitis B disease have symptoms. Only those with hepatitis B virus transmit the hepatitis D virus (HDV). An preexisting long-term (chronic) hepatitis B illness is worse than a current (acute) hepatitis B disease caused by HDV. Even those who have the hepatitis B virus yet have never experienced symptoms may develop symptoms as a result of it. The use of intravenous (IV) or injectable medications, being contaminated while expecting (the mother may transmit the virus to the fetus), having the hepatitis B virus, engaging in sexual activity with other males, coupled with getting a lot of blood transfusions are risk factors. Abdominal discomfort, dark urine, exhaustion, joint pain, lack of appetite, coupled with vomiting are a few symptoms that might be present.

E. Hepatitis E

Hepatitis E is a disease with the hepatitis E virus that results in liver inflammation. The virus is among the five recognized human hepatitis viruses. Assertions A through E The hepatitis E virus seems to be an icosahedral, positive-sense, single-stranded RNA virus. HEV are primarily transmitted through fecal pollution of water meant for drinking caused by poor sanitation (World Health Organization, Hepatitis E Fact Sheet, 2017; Khuroo *et al*., 2016). eating tainted food, including raw otherwiise undercooked animal flesh, may spread an infection to humans, is another mode of transmission (Coloson *et al.*, 2010; Lewis *et al*., 2010; Chijiwa *et al*., 2005; and Martelli *et al*., 2012) coupled with by the transfusion of contaminated blood components, that is more frequent in places with a high endemicity (Khue *et al.,* 2004). Jaundice, lethargy, nausea, purging, stomach discomfort, hepatomegaly, as well as arthralgia are a few possible symptoms..

2.2. 4 Alagille Syndrome (Kamath ***et al***., 2007)

Alagille syndrome may be inherited through one affected parent and are occasionally an autosomal dominant condition. Additional times, a gene mutation happens on its own, which means neither parent has a copy of the altered gene. Alagille syndrome does have a 50% risk of occurring in a kid if one of the parents has it. The Jagged1 (JAG1) gene is often mutated or defective in individuals with Alagille syndrome. Less than 1% of persons with Alagille syndrome have mutations in the NOTCH2 gene. In the first few weeks, children with Alagille syndrome might exhibit liver disease symptoms coupled with inadequate bile outflow from the liver. Alagille syndrome may cause these symptoms across both children coupled with adults.

2.2.5 Alcohol-Related Liver Disease (O'Shea *et al*., 2010; Choi *et al*., 2012)

Liver cells may be destroyed otherwise damaged by alcohol. The liver breaks down alcohol in order for the body can get rid of it. If you consume more alcohol than your body can handle, your liver might suffer catastrophic harm. Alcoholic cirrhosis, alcoholic hepatitis, coupled with fatty liver disease were the three primary kinds of alcohol-related liver illness. Over time, fatty liver illness, alcoholic hepatitis, coupled with alcoholic cirrhosis are common progressions in problem drinkers. Nevertheless, not all heavy drinkers will have alcoholic hepatitis before developing cirrhosis. Some individuals with alcoholic hepatitis might not even exhibit any symptoms.

2.2.6 Gilbert's Syndrome (Claridge LC *et al*., 2011)

Gilbert's syndrome is now a typical, mild liver disease where the bilirubin that the liver produces is improperly processed. Bilirubin is produced by the breakdown of red blood cells. Gilbert's condition is often not hazardous and requires no medical attention. Gilbert's syndrome is caused by a genetic gene mutation. Gilbert's syndrome is a condition that frequently stays unidentified for a long time. For instance, Gilbert's syndrome is commonly discovered by accident whenever a people's blood test results show elevated levels of bilirubin. Other names for Gilbert's condition include constitutional hepatic dysfunction coupled with the increasingly prevalent non-hemolytic jaundice.

2.2.7 Nonalcoholic Steatohepatitis (American Liver Foundation, 2006)

NASH, also known as non-alcoholic steatohepatitis, would be a prevalent and frequently "silent" liver condition. It resembles alcoholic liver damage despite the fact that occurs in people who drink less to no alcohol. Fat inside the liver, coupled with inflammatory response coupled with destroyed, is the main symptom of NASH. Most NASH sufferers are in good health coupled with are unaware of their liver issues. NASH may, however, progress to cirrhosis, a condition where the liver is scarred for life coupled with damaged coupled with is no longer capable of functioning normally. While possessing fat inside the liver is abnormal, it certainly doesn't permanently injure the organ. Nonalcoholic fatty liver disease is the term used when fat is detected as a consequence of blood test results or liver imaging (NAFLD). A liver biopsy in this situation will reveal which some patients have NASH coupled with others only possess a fatty liver.

2.3 LIPID PERIOXIDATION AND FREE RADICALS

According to several studies, the primary cause of liver failure, including hepatocyte deterioration, necrosis, edema, as well as apoptosis, is oxidative stress brought on by free radicals. Free radicals often cause liver harm or damage by lipid peroxidation, a covalent link, which leads to further tissue harm. ROS, those include peroxyl, hydroxyl, as well as alkoxy, along with superoxide radicals, serious harm the lipids, proteins, as well as nucleic acid inside the membrane. This was also connected to a number of ageing process problems, such as atherosclerosis, lung as well as kidney malfunction, cancer, inflammatory illness, but also cardiovascular problems (Pal et al., 2014; Singh et al., 2008). Lipid peroxidation disrupts cell membranes, and that in turn damages the membrane's structural stability and functioning. This has an adverse effect on the cell's capacity to keep steady ion gradients as well as transport ions (Madkour and Abdel-Daim, 2013) But at the other side, excessive drug usage exposure to certain toxins may potentially harm the liver (Haque *et al*., 2014).

A set of oxidative lipid breakdown events make up lipid peroxidation. Free radicals "steal" electrons through the lipids within cell membranes during this procedure, instigating damages the cells. The action being driven by a free radical chain reaction mechanism. Due to the many double bonds as well as methylene bridges (-CH2-) they comprise, polyunsaturated fatty acids are the ones that are typically affected due to their particularly reactive hydrogen atoms. End products of lipid peroxidation include reactive aldehydes including malondialdehyde (MDA) as well as 4-hydroxynonenal (HNE), the latter of thats is likewise known as "the second messenger of free radicals" as well as a main bioactive marker of lipid peroxidation due to their many biological activities of reactive oxygen species (Satish and Dilipkumar, 2015).

2.3.1 Hydroxyl radicals (OH^-)

These radicals were extremely reactive, effortlessly forming hydroxyl groups, also thus have a short half-life. According to reports, the hydroxyl radical (OH) as well as other radical species hit each cell of human an average of 105 instances per day, causing oxidative stress (Valko *et al.,* 2004; Carocho and Ferreira, 2013). On rare occasions, immunological response might result in the production of hydroxyl radicals. This chemical is primarily produced by microglia as well as macrophages in response to very particular infections as well as certain microorganisms.

Significance: The Many neurological autoimmune illnesses have been linked to the harmful activity of OH, which occurs whenever immune cells becoming overactive and toxic towards nearby healthy cells (Gulcin 2012).

2.3.2 Superoxide Ion Radical

The autooxidation reaction, an enzymatic process, and a nonenzymatic electron transfer reaction that involves the electrons are transferred from molecular oxygen lead to the formation of the superoxide anion radical, one of the most significant and prevalent ROS (Michelson *et al., 1977).* Its level of reactivity between biomolecules is minimal as well as it is primarily synthesized inside the mitochondria. The enzymes xanthine oxidase, cyclooxygenase, as well as NADPH dependency oxidase were accomplished of producing superoxide. It may take one of two shapes including as O2•− or hydroperoxyl radical (HO_2) at low pH (Bielski and Cabelli, 1996). The much more significant form would be the hydroperoxyl radical, which might enter the phospholipid bilayer greater readily than the charged form (O2•). Superoxide the most

prevalent type at physiological pH levels. Additionally, it has the ability to oxidize substances like tocopherol as well as ascorbic acid.

2.4 ANTIOXIDANTS

A chemical is considered an antioxidant if it considerably slows down or stops the oxidation of a substrate (such as DNA, proteins, lipids, or carbohydrates) while it is available at a lower concentration than the substrate (Rice-Evans, 1998). An imbalance among the generation of ROS and antioxidant defences was used to characterize oxidative stress (Buldurun et al. 2020). Free radicals, which seek stability by electron pairing between biological macromolecules including proteins, lipids, and DNA in healthy human cells, are the source of oxidative stress. These molecules also cause lipid peroxidation, protein degradation, and DNA damage (Hazra et al., 2008). Even though oxidation process are essential for life, those who may also be harmful. As a result, both plants and animals have complex systems that contain many different antioxidants, including glutathione, vitamin C, coupled with E, as well as enzymes like catalase, in addition tonumerous perioxidases (Halliwell and Gutteridge, 2014). By eliminating the free radical intermediates thus preventing additional oxidation events, antioxidants break these chain reactions. In recent times, antioxidant chemistry has received a great deal of attention as owing to its threats posed to biological macromolecules through oxidative processes (Sies, 2017). Plants are the major focus of researchers for the isolation of antioxidant secondary metabolites (Koleva *et al*., 2012).

2.4.1 Superoxide Dismutase (SOD): SOD constitutes a group of enzymes that catalyze the dismutation of the superoxide anion, a highly reactive free radical, into oxygen coupled with hydrogen peroxide (Ozolua *et al*., 2019). As a result, they constitute an essential component of

every cell's antioxidant defence mechanism. Extracellular SOD, according to investigations, is essential in the battle of oxidative stress-related pathophysiologies like ischemia reperfusion damage, hypertension, and lung wound (Aziz *et al*., 2019).

2.4.2 Malondialdehyde (MDA): MDA occurs naturally in tissues as a product of perioxidative changes. Free radicals generated by metabolic processes are capable of causing alterations in the MDA level in tissues; thus, the level of MDA in tissues act in an essential role in assessing the extent of perioxidative damage that has occurred in tissues (Ozolua *et al*., 2019).

2.5 ASCORBIC ACID (VITAMIN C)

A monosaccharide oxidation-reduction (redox) catalyst known as ascorbic acid, often known as vitamin C, was present inside both plants coupled with animals (Linster, 2007). Since humans have to get ascorbic acid via its food, it is considered a diet vitamin since one of the enzymes required to produce it was lost via mutation throughout monkey evolution. The majority of other animals could make this substance internally and do not necessitate it in its meals (Linster, 2007).

Significance: Reactive oxygen species like hydrogen peroxide may be neutralized through reducing ascorbic acid, a redox catalyst. According to reports, ascorbic acid has powerful free radical scavenging properties towards $O2\cdot-$, H_2O_2, $OH\cdot$, O2, and reactive $NO2\cdot$ (Barros *et al*. 2011).

2.6 PHENOLIC COMPOUND

The flavonoids, tannins, lignans, phenolic acids, as well as terpenes are the most prevalent plant phenolic chemicals (Nazck and Shahidi, 2006; Shahidi and Ambigaipalan, 2015). Almost all plant products, particularly meals made from plants, naturally contain phenolic compounds as secondary plant metabolites. According to Gülçin (2006), these substances are regarded as essential components of both the human coupled with animal diets. It is commonly recognised that the location as well as amount of -OH groups as well as the kind of replacements on the aromatic rings affect the antioxidant capacity of phenolic compounds (Cosme *et al.,* 2018).

A. Phenolic acid

Phenolic acid constitutes approximately thirty percent of the dietary phenols found inside plants in free as well as bound form (Robbins, 2003). A benzoic acid acid group otherwise the cinnamic acid group gives birth to aromatic carboxylic acids, that are hydroxy derivatives of phenolic acids. Phenolic acids, including *p*-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acid, chlorogenic acid, in addition to rosmarinic acid, are extensively scattered across the plant kingdom (Öztürk Sarikaya *et al*. 2010).

B. Flavonoids

Flavonoids are a sizable category of polyphenols that are normally present substances that are widely present inside plant-created diets. The two benzene rings (A and B) of the fundamental structure exhibit a variety of substitution sequences throughout nature (Ghosh *et al*. 2015). The majority of flavonoids inside the human diet were found within foods like fruits, vegetables, plus drinks made from plants like tea. A few hundred milligrams of flavonoids should be

consumed daily. Approximately 4000 distinct contains natural flavonoids were identified up to this point (Ghosh *et al*., 2015).

Significance: It's been suggested that flavonoids, which are potent antioxidants, prevent heart diseases by preventing the oxidation of low-density lipoproteins. Additionally, flavonoids are one of our diet's main sources of antioxidants. The metal-chelating potential of flavonoids, that is heavily affected by the configuration of hydroxyl as well as carbonyl groups mostly around molecule, the inclusion of hydrogen otherwise electron donating substituents that can neutralize free radicals, as well as the capacity of the flavonoid towards delocalize the unpaired electron, resulting in the production of a stable phenoxy radical are all factors that affect the effectiveness of flavonoids as antioxidants (Gulcin *et al*., 2011; Gulcin, 2012).

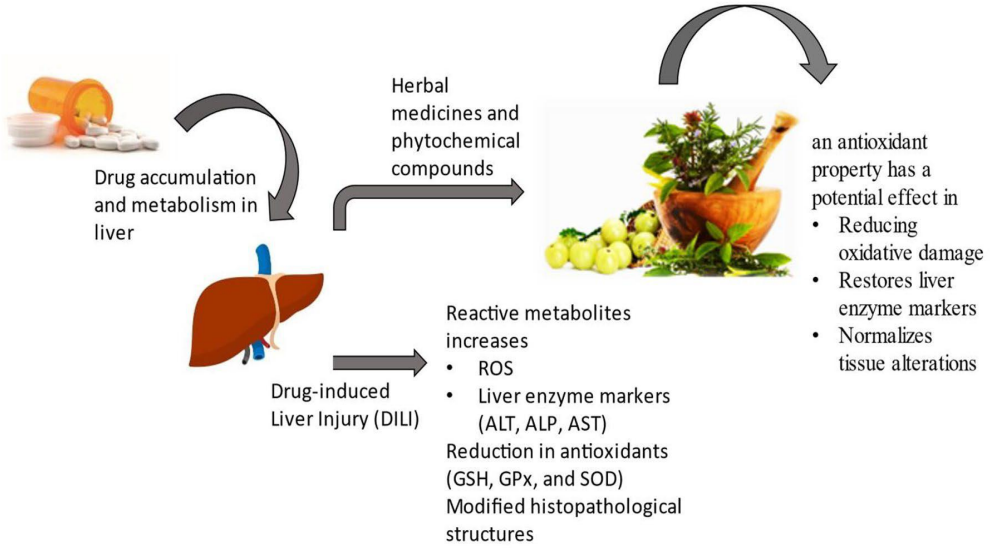
C. Quercetin: Quercetin is indeed a polyphenolic family of flavonoids that is present predominantly as glycosides inside a variety of fruits and vegetables, including citrus fruits, cherries, grapes, and asparagus, as well as other foods like cocoa, whole grains, coupled with green otherwise black tea (Andres *et al*., 2018).

D. Tannins: Tannins were classified as either condensed otherwise hydrolyzable proanthocyanidins based on its chemical structure. Conversely, green vegetables, berries, as well as legumes were its main supply of hydrolyzable tannins (Shahidi and Naczk, 2004; Shahidi and Ambigaipalan, 2015).

Significance: Tannic acid can also be seen to have powerful H2O2 scavenging, superoxide, DPPH, as well as ABTS radical scavenging, Fe3+ reducing, as well as metal chelation on ferrous ion actions (Gulcin *et al.*, 2010).

2.7 MEDICINAL PLANT ALTERNATIVE FOR TREATMENT

Lately, less hazardous plant-based chemicals have been used as hepatoprotective medications. Therefore, subsequent research throughout this sector has focused on searching plant diversity for new hepatoprotective capabilities. (2008) Afaf et al. But prior research has shown that excessive ROS generation exacerbates oxidative stress, culminating inside an injury mechanism linked to prevalent clinical disorders as hyperglycemia, kidney in addition to liver damage, cancer, as well as heart problems; (Jaeschke, 2011) In order to avoid oxidative stress damage, it's essential to keep the equilibrium among ROS and antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px). 2007; Yen et al. According to Kamalakkannan et al. (2005), several natural substances as well as medicinal plants shown protective benefits versus liver damage brought on by CCl4, and in numerous instances, this protective reaction was brought on through its antioxidant characteristics. (2000) Lin et al. Alternative treatments for hepatotoxicity that use natural therapies made by medicinal plants are said to be the most popular as well as exciting (Fakurazi et al., 2012) Plants that are high in natural antioxidants, such as flavonoids, tocopherol, vitamin A, C, as well as E, as well as various phenolic compounds, have been shown to have hepatoprotective properties (Sabir and Rocha, 2008).



(Source: Manisha and Sabina, 2021).

Figure 2: Liver injury and protective effect of herbal plants.

Table 1: Medicinal plants with hepatoprotective potentials (Okalyeto *et al*., 2018)

S/N	Family	Name of plant	Plant parts used	Extract used	Oral dose (mg/kg)	Hepatotoxicity inducing agents	Biochemical parameters studied
1	*Euphobiaceae*	*Phyllanthus muellariarus*	Leaves	Aqueous	400 mg/kg	Acetaminophen	ALP,ALT,AST,ALB,TB, CAT,SOD,GSH
2	*Lamiaceae*	*Thymus linearis*	Leaves	Aqueous/ ether	250 & 500 mg/kg	PCM & CCl_4	ALT, AST, ALP, TB, SGOT & SGPT
3	*Lamiaceae*	*Ocimum gratissimum*	Fresh leaves	Methanol	40 mg/kg	CCl_4	ALT, AST & ALP.
4	*Vitaceae*	*Vitis vinfera*	Roots	Ethanol	200 mg/kg	CCl_4	SGPT, SGOT, ALP & TB.
5	*Zingiberaceae*	*Curcuma longa*	Rhizome	Ethanol	600 mg/kg	PCM	ALT, ALP & AST

2.8 LIVER FUNCTION TESTS

A variety of liver function tests, including those for blood proteins, blood albumin, direct coupled with indirect bilirubin, ALT, AST, GGT, ALP, PT, in addition to PTT, are essential in determining if the liver is functioning properly. The liver tissue coupled with bile ducts could be examined using imaging techniques including magnetic resonance imaging, ultrasonography, as well as transient elastography. To study liver tissue and identify various disorders, a liver biopsy may be conducted; however, in certain circumstances, procedures like elastography might eliminate the requirement for a biopsy (Tapper and Lok, 2017). The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), as well as gamma-glutamyl transferase (GGT) have historically been utilized in clinical settings to identify liver injury. These biomarkers quantify either shifts in tissue as well as cell integrity or an alter inside normal liver activity (Aithal et al., 2011; Andrade et al., 2019). Because the large bulk of drug-induced liver damage develop in a unique way, biomarkers are especially helpful throughout this circumstance (Larrey *et al*., 2017; Andrade *et al*., 2019).

2.7.1 Alanine Amino Transaminase (ALT): Alanine amino transaminase (ALT), also referred to as Serum Glutamate-oxaloacetate transaminase (SGOT). Alanine aminotransferase (ALT) are an enzyme found within hepatocytes (liver cells) as well as to a leser extent in kidney, heart and muscles (Ozolua *et al*., 2019). This enzyme seeps from injured cells right into the blood, which is detected. Acute liver damage caused by viral hepatitis or paracetamol (acetaminophen) abuse causes ALT to increase sharply. Numerous multiples of the upper limit of standard Reference are frequently employed to evaluate elevations: Serum up to 12U/L (Schmidt, 2013).

Functions of ALT: It catalyzes the transfer of an amino group from alanine to a-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate (Schmidt, 2013).

Clinical Significances: It is often tested clinically to measure the health of a liver as component of a diagnostic liver function test. For diagnostic purposes, it is often expressed in units per litre (U/L) (Schmidt, 2013). Hugely increased ALT values often point to the presence of additional medical conditions including myopathy, congestive heart problems, viral hepatitis, liver injury, bile duct issues, and infectious mononucleosis. Because of this, ALT is often used to check for liver issues. Elevated ALT values, though, may not always indicate the presence of health issues. ALT levels fluctuate naturally during the duration of the day, in addition they may also rise in reaction to vigorous activity (Omata *et al*., 2016).

Reference values: Human: Adult males (10-40 U/I); adult females (7-35 U/I); newborns (13-45 U/I), Rats: (40-105 U/I) (Ozolua *et al*., 2019).

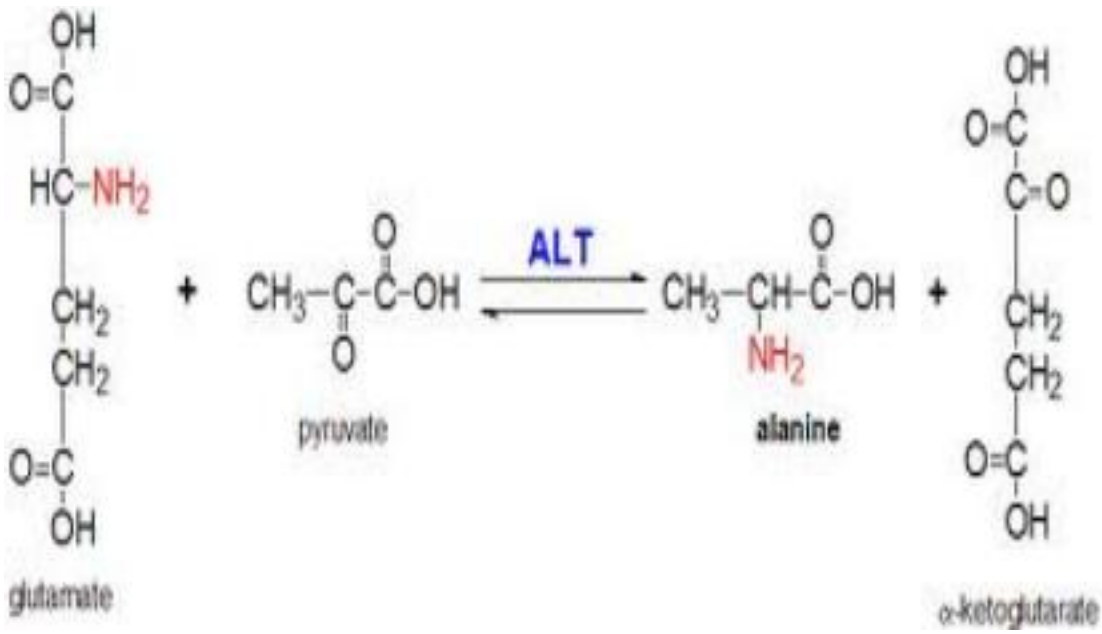


Figure 3: Reaction Mechanism of ALT Source**:** (Schmidt, 2013).

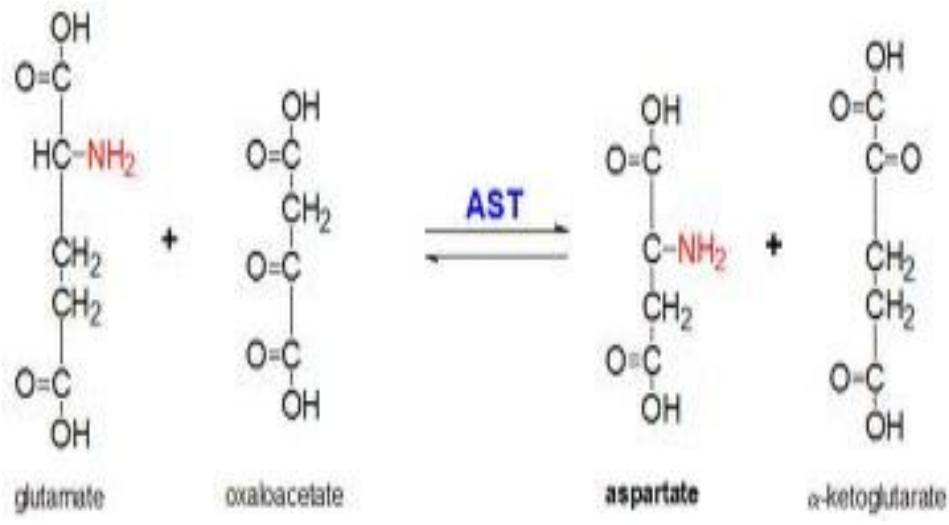
2.7.2 Aspartate amino Transaminase (AST): Comparable to ALT, aspartate amino transaminase (AST) are an enzyme connected to liver parenchymal cells. Although it is elevated in cases of acute liver injury, it also found throughout red blood cells, cardiac muscle, as well as skeletal muscle, thus it is not exclusive to the liver (Paul and Giboney, 2008). ALT is mostly found inside the liver, with smaller amounts also being present inside the kidneys, heart, as well as skeletal muscle. The AST could also be raised in common infections other organs, including the heart or muscles throughout myocardial infarction, as well as in acute pancreatitis, acute hemolytic anemia, severe burns, acute kidney problems, musculoskeletal conditions, and trauma. As a direct consequence, the ALT is a higher accurate measure of liver inflammation than the

AST (Kondo, Wakabayashi, Yagi and Kagamiyama, 2014). Often it helps to distinguish between the various types of liver injury by looking at the proportion of AST to ALT. Increased AST levels were also utilized as a cardiac marker, therefore they are not just associated with liver injury (Gaze, 2007).

Clinical Significances: Particularly high levels of aspartate amino transaminase indicate alcohol-related harm. Alanine amino transaminase (ALT) as well as aspartate amino transaminase are the two transaminases that are most often measured during liver testing (AST). They are probably present if the liver is injured (nonetheless, their existence is a symptom of major issues and does not always indicate liver damage). The crucial metric is the similarity of ALT as well as AST levels. In whatsoever disease condition, an AST is comparatively lesser than an ALT. If the AST is higher, particularly double or triple of the ALT, is generally indicating alcoholic liver disease (Omata *et al.,* 2016). It speeds up the transformation of glutamate and oxaloacetate from aspartate as well as alpha-ketoglutarate, and conversely (Inoue *et al.*, 2011).

Reference values: Men (14-20 U/L, <35 U/L); Women (10-36 U/L, <31 U/L); Children (9-80 U/L); Newborns (47-150 U/I). Rats (140-225 IU/I) (Ozolua *et al*., 2019).

Figure 4:



Reaction Mechanism of AST Source**:** (Schmidt, 2013).

2.7.3. Alkaline Phosphatase: Alkaline phosphatase (ALP), a hydrolase enzyme, is responsible for removing phosphate groups from a range of substances, including proteins, nucleotides, as well as alkaloids. The process of removing the phosphate group is known as dephosphorylation. As their name suggests, alkaline phosphatases work much better inside an alkaline environment. It and basic phosphatase are used alternately on occasion. Alkaline phosphatase is found in all bodily tissues in humans, although it is notably abundant in the liver, kidney as well as placenta. (Lange *et al*., 2012; Giboney, 2015).

Function: ALP hydrolyses the substrate p-nitrophenol phosphate, liberating p-nitrophenol and inorganic phosphate (Rec, 1972).

Clinical Significance and Interpretation: High ALP levels indicate clogged bile ducts. In pregnant women as well as toddlers, concentrations are much greater. Also, as ALP is a consequence of osteoblast effect (as in Paget's disease of the bones), high ALP shows that active bone resorption could be occurring. Untreated Celiac Disease patients also have higher amounts. By employing electrophoresis during isoenzyme investigations, elevated levels may be verified. Bone as well as liver isoenzymes may be distinguished by their heat stability. Placental alkaline phosphatase is elevated in S.E.M.inomas and active form of Rickets (Lange *et al*., 2012). In conditions like hypophosphatasia and in postmenopausal women on estrogen treatment, lower levels of ALP are seen. Men who have just had cardiac surgery, are malnourished, magnesium deficient, hypothyroid, or have chronic anemia, Children suffering achondroplasia, pernicious, aplastic anemia, leukemia, as well as Wilson's disease. Children who have recently had a serious bout of enteritis. Oral contraceptives has been established to decrease alkaline

phosphatise (Schiele, Vincent-Viry, Fournier, Starck and Siest, 2018). Measurement of ALP is important in examination of two conditions: hepatobiliary illness and bone illness related to the development of new bone (Fischbash and Dunning, 2015; Burtis, Ashwood and Bruns, 2012; Ozolua *et al*., 2019).

Reference values: Males 4-15 years (54-369 U/I); males 20-30 years (53-128 U/I); males >60 years (56-119 U/I). females 4-15 years (54-369 U/I); females 20-50 years (42-98 U/I); females>60 years (53-141 U/I). Rats (230-360 IU/I) (Ozolua *et al*., 2019).

.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Plant Collection

Lemon was brought from New Benin Market in Oredo Local Government Area. Garlic, turmeric and ginger were purchased at Oriegbuni Market in Ikpoba Okha Local Governement Area. *Moringa olifera* leaf was gotten from farm land in Ordeo Local Government Area all in Edo State.

3.2 Preparation of plant materials

Lemon, garlic, turmeric and ginger were washed and chopped into smaller bits. The *Moringa olifera* leaf was removed from the stalk and washed. The chopped lemon, garlic, turmeric and ginger, the washed moringa leaves were dehydrated using a dehydrator. After dehydration, the dehydrated lemon, garlic, turmeric, ginger and moringa leaf were grounded to powder separately using impact mill. The powdered lemon, garlic, turmeric, ginger and moringa leaf were weighed and mixed in an equal proportion (1:1:1:1:1) to formulate the herbal tea. The herbal tea was formulated in such a way that 1g in a tea bag contained 200 mg of each powdered plant material.

3.3 Experimental Animals

Albino rat weighing 100-200g were purchased from animal house of Department of Pharmacology&Toxicology, Faculty of Pharmacy, University of Benin. The rats were housed within the animal facility of Department of Science Laboratory Technology and given two weeks acclimatization, under normal laboratory conditions with 12 hours light/dark cycle. They were fed with normal animal pellet and water spontaneously. The animals were handled in accordance with normal protocols for laboratory animals. (National Institute of Health USA Public Health Service Policy on Humane Care and Use of Laboratory Animals 2002).

3.4 Nutritional Composition

3.4.1 Proximate analyses

The proximate content such as moisture, ash, crude protein, ether extract, crude fibre and NFE of the formulated herbal tea was analyzed using method described by AOAC. (2000), Isikhuemen *et al*. (2020) and Noureddini and Byun. (2010).

3.4 2 Moisture Content Determination

Moisture was determined by the loss in weight that occurs when a sample was dried to a constant weight in an oven. One gram (1g) of the formulated herbal tea was weighed into a silica dish previously dried and weighed. The sample was then dried in an oven for 65^0C for 36 hours, cooled in a desiccator and weighed. The drying and weighing continues until a constant weight was achieved.

$$\%\text{Moisture} = \frac{\text{wt of sample} + \text{dish before drying} - \text{wt of sample} + \text{dish after drying}}{\text{Wt of sample taken}} X100$$

3.4.3 Ash

Ash is the inorganic residue obtained by burning off the organic matter of foodstuff at 400-600^C in muffle furnace for 4hrs. Two (2 g) of the formulated herbal tea was weighed into a pre-heated crucible. The crucible was placed into muffle furnace at 400-6000C for 4hrs or until whitish-grey ash was obtained. The crucible was then placed in the desiccator and weighed

$$\%\text{Ash} = \frac{\text{wt of crucible} + \text{ash} - \text{wt of crucible}}{\text{wt of sample}} X10$$

3.4.4 Fat and Oil

The flask contained 150 ml of anhydrous diethyl ether (petroleum ether), which has a boiling point between 40 and 600 C. Weighing 2 to 5 g of the prepared herbal tea sample into a thimble and plugging it with cotton wool. The extractor was filled with the filled thimble, and after that, the heated ether in the flask. The ether vapour condenses into liquid form as it passes through the extractor's side arm and falls back into the sample in the thimble. The ether-soluble compounds were then dissolved and brought back into solution through the siphon tube into the

flask. At least four hours of extraction are required. The thimble was taken off, and the extractor received the majority of the solvent that had been distilled from the flask. The flask was then unplugged, put in a 650°C oven for 4 hours, allowed to cool in a desiccator, and weighed.

$$\%\text{Fat and Oil} = \frac{\text{wt of flask} + \text{extract} - \text{tare wt of flask}}{\text{wt of sample}} X\ 100$$

3.4.5 Crude Fibre

To estimate the crude fibre, analyze the organic residue that remains following supplement extractions sequentially with ether. After transferring the fat-free material into a flask or beaker, 200 ml of preheated 1.25% H2SO4 was added. The solution was then gently boiled for around 30 minutes, with the acid volume being kept constant by the addition of hot water. By adding hot water to the Buckner flask funnel, which has a Whatman filter attached, the funnel was preheated. After boiling the acid sample combination, it was hotly filtered through the funnel with enough suction. After numerous rounds of boiling water washing (until the residue was neutral on litmus paper), the residue was placed back into the beaker. Then 200 ml of preheated 1.25% Na2SO4 was added, and the mixture simmered for an additional 30 minutes. Filter while being suctioned, then wash twice with ethanol and in hot water. The residue was weighted after drying at 650C for around 24 hours. The residue was deposited into a crucible, heated to 400–600°C in a muffle furnace, then allowed to cool in a desiccator before being weighed.

$$\%\ \text{Crude fibre} = \frac{\text{Dry wt of residue before ashing} - \text{wt of residue after ashing}}{\text{wt of sample}} X\ 100$$

3.4.6 Crude Protein

Crude protein was determined by measuring the nitrogen content of the formulated herbal tea sample and multiplying it by a factor of 6.25. This factor was based on the fact that most protein contains 16 % nitrogen. Crude protein was determined by **kjeldahl method.** The method involves: Digestion, Distillation and Titration.

3.4.7 Digestion: Two (2 g) of the formulated herbal tea sample was weighed into kjeldahl flask and add 25 ml of concentrated sulphuric acid, 0.5 g of copper sulphate, 5 g of sodium sulphate and a speck of selenium tablet. Heat was applied in a fume cupboard slowly at first to prevent undue frothing, continue to digest for 45 mins until the digesta become clear pale green. Leave until completely cool and rapidly add 100 ml of distilled water. The digestion flask was rinse 2-3 times and the rinsing was added to the bulk.

3.4.8 Distillation: Markham distillation apparatus was used for distillation. The distillation apparatus was steam up and add about 10 ml of the digest into the apparatus via a funnel and allowed it to boil. Ten (10 ml) of sodium hydroxide was added. Distil content was placed into 50 ml of 2 % boric acid containing screened methyl red indicator.

3.4.9 Titration: Alkaline ammonium borate formed was titrated directly with 0.1 *N* HCl. The titre value which was the volume of acid used was recorded. The volume of acid used was fitted into the formula which becomes

$$\%\mathrm{N} = \frac{14 \text{ x VA x } 0.1 \text{ x} X\mathrm{w} \text{ x} 100}{1000\mathrm{x}100}$$

VA = volume of acid used w= weight of sample

% crude protein = % N x 6.25

3.4.10 Nitrogen Free Extract (NFE)

NFE was determined by mathematical calculation. It was obtained by subtracting the sum of percentages of all the nutrients already determined from 100.

% NFE = 100 - (% Moisture + % CF + % CP + % EE + % Ash)

NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in the sample.

3.5 Mineral element determination

The determination of mineral contents was carried out using Atomic Absorption Spectrophotometer (AAS model: SOLAAR 968 Unicam Series) for Ca, Fe, Mg, Mn, Cu, Pb, Cr, **Se** and Zn; Flame Photometer for Na and K and Spectrophotometer (model: Spectronic 20D+) for P followed methods described by AOAC (2003).

For wet digestion of samples, 1.0 g of the powdered sample was weighed in digestion flask. 12 ml of concentrated HNO_3 was added and kept overnight at room temperature. 4.0 ml of $HClO_4$ was added to the mixture and heated in digestion block; starting at 50°C and gradually increasing to 250°C. The appearance of fumes at 70 – 80 min signaled completion of digestion. The mixture was allowed to cool before transferring into 100 ml volumetric flask and thereafter made to mark with distilled water. The wet digested solution was stored in plastic reagent bottle for use in determination of minerals following the principles and procedures expounded by Gul and Safdar (2009).

3.6 Characterization of Alkaloid,Tanning, Phenolic Compounds and Steroid

Quantitative and characterization of some phytochemical such as flavonoid, alkaloid, phenolic, tannin and steroid in the formulated herbal tea was determine using HPLC (Model:2000 Searchtech Instrument. UK).

3.6.1 Preparation of Standard Solutions

Stock solutions of the reference compounds was made by weighing 0.001g of reference standards for flavonoid, alkaloid, phenolic and tannin each into a test tube and dissolving each standard with 10 ml of 70 % methanol. Each of the standard was then agitated for 10 minutes using vortex mixer and then filtered using a cosmonice filter or micron filter into the sample bottle.

3.6.2 Determination of Phenolics

Chemicals and Materials

Glacial Acetic acid (analytical grade), methanol (lichrosolv), acetonitrile (lichrosolv), ascorbic acid, gallic acid, catechin, methy gallate, caffeic acid, syringic acid, ellagic acid, chlorogenic acid (analytical grades) reference standards.

Sample Preparation and Extraction

Sample extract (0.1g) was dissolved in 10 ml of 70% methanol and allowed to stand for 1 to 2 hours in a closed test tube. The extracted sample was then decanted, centrifuged and filtered using a cosmonice filter or micron filter into a 5ml sample bottle.

Procedure for Analysis

1. Mobile phase was prepared by mixing acetonitrile,Water and acetic acid (19:80:1).
2. The wavelength of the HPLC was set at 272 nm.
3. Column temperature was set to 40 ^{0}C.
4. Run time was set at 25 minutes.
5. Sample volume of 40 micro liters was injected into injector of the HPLC.
6. The mobile phase was pump in which allowed the sample to be carried into the column
7. The chromatogram was obtained from the display system after the run time.
8. Standard was prepared by using reference reagents listed above

9. The retention time of the standard was compared with that of the chromatogram obtained from the sample to determine the phenolics content in the sample

3.6.3 Determination of Alkaloids

Chemicals and Materials

Methanol (lichrosolv), acetonitrile (lichrosolv), nicotine, anatabine, cotinine, anabasine, and myosmine (analytical grades) reference standard.

Sample Preparation and Extraction

Sample (0.1g) was dissolved in 10ml of 70% methanol and allowed to stand for 1 to 2 hours in a closed test tube. The extracted sample was then decanted, centrifuged and filtered using a cosmonice filter or micron filter into a 5 ml sample bottle.

Procedure for Analysis

1. Mobile phase was prepared by mixing methanol, acetonitrile and water (70:20:10)
2. The wavelength of HPLC was set at 260 nm.
3. Column temperature was set to 40 ^{0}C
4. Run time was set at 15 minutes.
5. Sample volume of 40 micro liters was injected into the HPLC injector.
6. The mobile phase was pump in which allowed the sample to be carried into the column
7. The chromatogram was obtained from the display system after the run time.
8. Standard was prepared using reagents listed as reference above

9. The retention time of the standard was compared with that of the chromatogram obtained from the sample to determine the alkaloid content in the sample

3.6.4 Determination of Tannin

Chemicals and Materials

Methanol, Tannic acid (analytical grade) reference standard.

Sample Preparation and Extraction

Sample extract (0.1g) was dissolved in 10ml of 70% methanol and was allowed to stand for 1 to 2 hours in a closed test tube. The extracted sample was decanted, centrifuged and filtered using a micron filter into a 5ml sample bottle.

Procedure for Analysis

1. Mobile phase was prepared by mixing mobile phase was methanol and water (50:50)
2. The wavelength the HPLC was set at 270 nm.
3. Column temperature was set to 40 ^{0}C.
4. Run time was set at 10 minutes.
5. Sample volume of 40 micro liters was injected in the HPLC injector.
6. The mobile phase was pump in which allowed the sample to be carried into the column
7. The chromatogram was obtained from the display system after the run time.
8. Standard was prepared using reagents listed as reference above

9. The retention time of the standard was compared with that of the chromatogram obtained from the sample to determine the tannin content in the sample

3.7 Hepatoprotective Experimental Design

Hepatoprotective activity was study according to the method of Mohammed, (2018). Albino rats 24 were allotted into 6 groups of 4 rats in each group as follows:

Group 1: Received distilled water

Group 2: Received 5mg/kg of extract of polyherbal formulation

Group 3: Received 10mg/kg of extract of polyherbal formulation

Group 4: Received 5mg/kg of extract of polyherbal formulation + CCl_4

Group 5: Received 10mg/kg of extract of polyherbal formulation + CCl_4

Group 6: Received CCl_4 alone

The animals were administered polyherbal extract orally for 4 days while CCl_4 1:1 in olive oil was administered twice a week. After 14 days' oral administration of extract, the animals were sacrificed using chloroform as an anesthesia. Blood was collected for biochemical and antioxidant analysis and each liver was weighed.

3.8 Biochemical analysis

3.8.1 Determination of Alkaline Phosphatase (ALP)

Alkaline phosphate substrate (0.5 ml) was pipetted into test tubes labeled blank, standard and sample respectively. 50ul of distilled water, standard and sample were added to test tubes respectively and were incubated for 10 minutes at 37^{o}c. Then, 2.5 ml of alkaline phosphatase

colour developer was added to labeled test tubes. The spectrophotometer was zeroed with reagent blank and absorbencies of all tubes were measured at a wavelength of 590 nm. The concentration of alkaline phosphate was calculated using the following formula:

Alkaline Phosphatase Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

3.8.2 Determination of Alanine Aminotransferase (ALT)

To 5 ml of each blood sample, 0.1 ml of solution RI (buffer) was added and mixed. It was incubated for 30 minutes at 37^0c. 0.5ml of solution R2 (2,4 – dinitrophenylhydrazine) was added and the mixture was allowed to stand for 20 minutes at room temperature after which 5.0ml of Sodium hydroxide was added. The process was repeated for all samples. The samples were read using a spectrophotometer at wavelength of 546 nm. The samples were read against a blank. The concentration of Alanine Aminotransferase (ALT) was calculated using the following formula:

Alanine Aminotransferase Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

3.8.3 Determination of Aspartate aminotransferase (AST)

To 5 ml of each of the blood samples 0.1 ml of solution RI (buffer) was added and mixed and incubated for 30 minutes at 37^oc. Solution R2; 2,4 – dinitrophenylhydrazine (0.5 ml) was added and the mixture was allowed to stand for 20 minutes at room temperature after which 5.0 ml of Sodium hydroxide was added. The process was repeated for all samples. The samples were read

using a spectrophotometer at wavelength of 546 nm. The samples were read against a blank. The concentration of Aspartate aminotransferase was calculated using the following formula:

Aspartate aminotransferase Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

3.8.4 Determination of Total Protein

To 0.01 ml of the blood samples, distilled water and standard was pipetted into separate test tubes and labeled sample, blank and standard respectively. To the test tube containing sample and standard 0.5 ml of solution RI was added, to the test tube 0.5 ml of solution R2 was added to the test tubes containing blank. The solution in the test tube was then mixed and incubated for 30 minutes at 25°c. The samples were read using a spectrophotometer at wavelength of 546 nm. The samples were read against the blank which contained both solutions and Sodium hydroxide and water for the absorbance. The concentration of total protein was calculated using the following formula:

Total Protein Concentration (u/L): = (Abs of sample / Abs of standard) x Concentration of standard (mg/dL)

3.9 Antioxidant Analysis

3.9.1 Determination of Superoxide Dismutase (SOD)

Carbonate buffer (2.5 ml) was measured into labeled test tubes. 0.2 ml of tissue homogenate was added to the test tubes. 0.2 ml of distilled water was also measured into reference test tube. 0.3 ml of 0.3 ml epinephrine solution was added to each of the test tubes and to the reference tube. They were properly mixed and read at absorbance of 420 nm every 30 – 120 secs with UV

spectrophotometer (Model T80+UV spectrophotometer, PG Instruments Ltd). Distilled water was used to zero the machine (Misra and Fridouich, 1972)

3.10 Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM) and 'n' represents the number of guniea pigs or mice per experimental group. One-way analysis of Variance (ANOVA) were performed with Newman Keuls' post hoc test. All data were analyzed using Graph Pad Prism (UK) software version 6. $P<0.05$ shows a significant difference between compared data.

CHAPTER FOUR

4.0 RESULT

4.1 Nutritional composition of polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric and lemon).

Table 2 shows the proximate composition of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) with carbohydrate being the most abundant nutrient present.

4.2 Mineral composition of polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric and lemon).

Table 3 shows Micro and macro mineral content of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) with potassium, calcium, magnesium, phosphorus and iron being the most abundant mineral content.

4.3 phytochemical analysis

4.3.1 Characterization of phenolic compounds

Figure 5 and table 4 shows the quantity of phenolic compounds present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon). It shows that Luteolin, Arbutin, Kaempferol, Apigenin and Quercetin were the most abundant phenolic compound present in the polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon) as characterized with HPLC.

4.3.2 Characterization of alkaloid compounds

Figure 6 and table 5 shows the quantity of alkaloids present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) with Quinine being the most abundant

alkaloid in polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon)**,** as characterized with HPLC.

4.3.3 Characterization of steroids compounds

Figure 7 and table 6 shows the quantity of steroids present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon). It shows that Diosgenin and Ergocalciferol were the most abundant steroid in the polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon)**.**

4.3.3 Characterization of tannins compounds

Figure 8 and table 7 shows the quantity of tannins present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon). It shows that Epigattotechin and Catechin were the most abundant tannins in polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon)**.**

4.4 Hepatoprotective effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) in CCL4induced hepatotoxicity.

4.4.1 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on body weight

Figure 9 shows that polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric and lemon) at 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 and CCL4 without treatment significantly reduced the body weight of the animals when compared to control (*$p<0.05$).

4.4.2 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on liver to body weight ratio in CCL4 induced hepatotoxicity in rat.

Figure 10 shows that polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) at 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 and given distilled water significantly reduced liver weight when compared with CCL4 without treatment (***$p<0.001$; *$p<0.05$) on CCL4 induced hepatotoxicity in rat.

4.4.3 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on ALP in CCL4 induced hepatotoxicity in rat.

Figure 11 shows that polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) at 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 and given distilled water significantly reduced the value of ALP in the animals when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$).

4.4.4 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on AST in CCL4 induced hepatotoxicity in rat.

Figure 12 shows that polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) at 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 and given distilled water significantly reduced the value of AST in the animals when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$).

4.4.5 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on ALT in CCL4 induced hepatotoxicity in rat.

Figure 13 shows that polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) at 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 and given distilled water significantly reduced the value of ALT in the animals when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$).

4.4.6 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on total protein in CCL4 induced hepatotoxicity in rat.

Figure 14 shows that polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) had no effect on total protein when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$) in CCL4 induced hepatotoxicity in rat.

4.4.7 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on Catalase level in CCL4 induced hepatotoxicity in rat.

Figure 15 shows that polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) at 5 mg/kg of the formulated extract and 5 mg/kg of the extract + CCL4 and given distilled water significantly increased Catalase level in CCL4 induced hepatotoxicity in rats when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$).

4.4.8 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on MDA level in CCL4 induced hepatotoxicity in rat.

Figure 16 shows that polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) at 5 mg/kg of the formulated extract and 5 mg/kg of the extract + CCL4 and given distilled water significantly reduced MDA level in CCL4 induced hepatotoxicity in rats when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$).

4.4.9 Effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on SOD level in CCL4 induced hepatotoxicity in rat.

Figure 17 shows the effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) at 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, and 10 mg/kg of extract + CCL4 and distilled water significantly increased SOD level in CCL4 induced hepatotoxicity in rat when compared with CCL4 without treatment (****$p<0.0001$; ***$p<0.001$; **$p<0.01$; *$p<0.05$).

Table 2: Proximate composition of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon).

Proximate content	Amount (%)
Carbohydrate	64.660±0.085
Fat	6.357±0.007
Moisture content	6.123±0.003
Ash content	0.240±0.006
Protein	19.250±0.000
Fibre	3.367±0.088

Values were presented as mean±S.E.M, n= 3

.

Table 3: Micro and macro mineral content of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon).

Micro and macro element	Amount (mg/kg)
Sodium	0.470±0.000
Potassium	1356.00±0.00
Calcium	821.30±0.00
Zinc	65.50±0.00
Copper	13.30±0.00
Iron	221.40±0.00
Magnesium	380.80±0.00
Phosphorous	331.40±0.00
Manganese	31.50±0.00
Lead	3.50±0.00
Chromium	0.27±0.00
Selenium	4.420±0.172

Values were presented as mean±S.E.M, n= 3

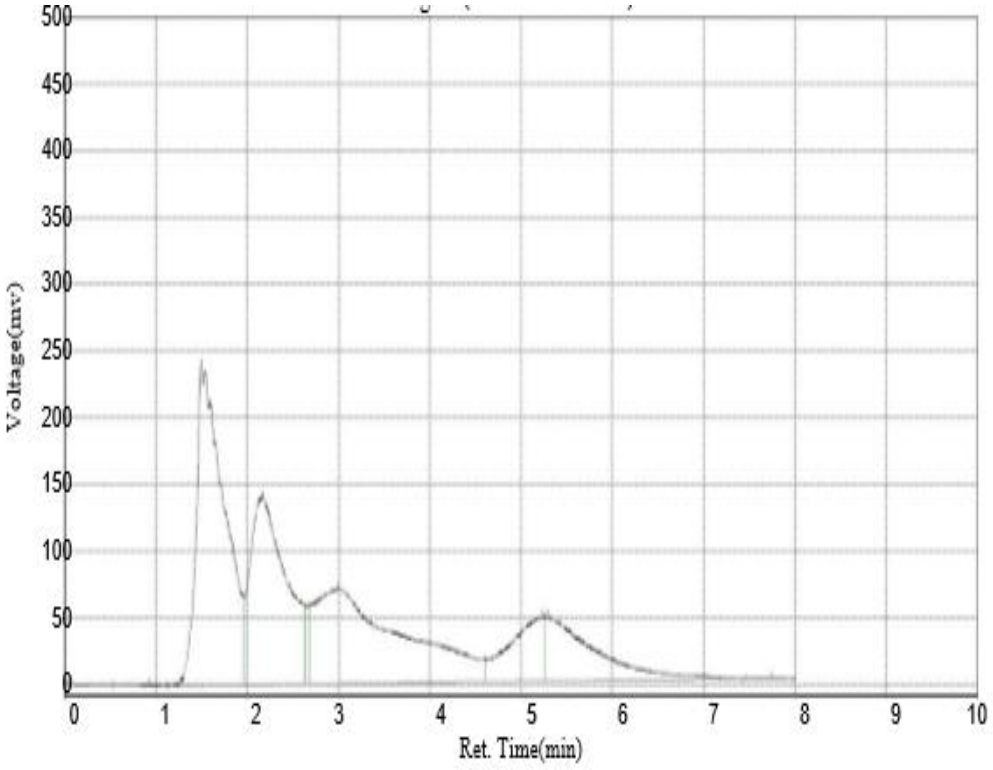


Figure 5: Chromatogram indicating phenolic compounds present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) as characterized with HPLC.

Table 4: Phenolic compounds in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) characterized with HPLC.

Peak No.	Peak ID	Ret Time	Height	Area	Conc mg/100g
1	Unidentified	0.065	591.758	1771.500	0.0106
2	Unidentified	0.923	206.667	3824.700	0.0228
3	Luteolin	1.490	238721.891	5134579.000	30.5885
4	Kaempferol	2.165	140273.922	3840852.000	22.8813
5	Arbutin	2.998	71292.656	4727025.500	28.1606
6	Quercetin	5.232	48297.945	1276431.000	7.6042
7	Apigenin	5.290	48165.676	1785297.750	10.6357
8	Naringnin	7.740	870.016	16184.300	0.0964

Luteolin, Arbutin, Kaempferol, Apigenin and Quercetin were the most abundant phenolic compound present in polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon)

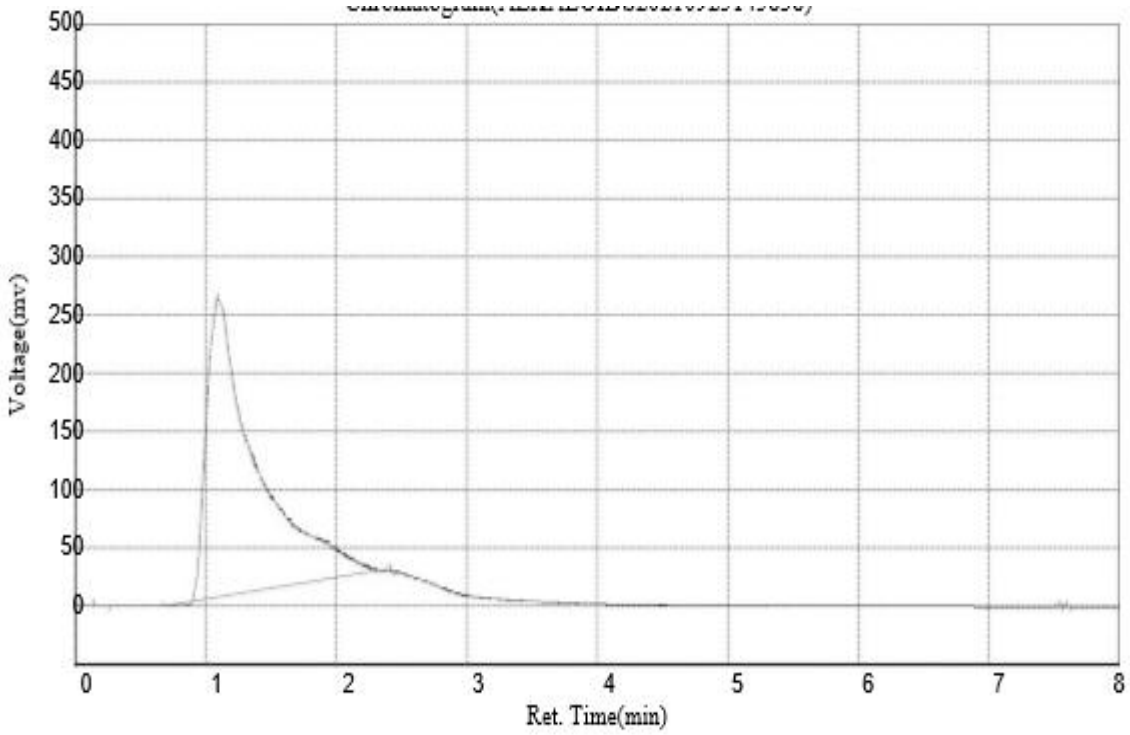


Figure 6: Chromatogram indicating alkaloids present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) as characterized with HPLC.

Table 5: Alkaloids in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) characterized with HPLC.

Peak No.	Peak ID	Ret Time	Height	Area	Conc mg/100g
1	Unidentified	0.132	269.500	987.300	0.0145
2	Quinine	1.090	255281.688	6819710.500	99.9669
3	Caffeine	1.807	776.429	1052.950	0.0154
4	Strychnine	7.040	67.385	99.954	0.0015
5	Unidentified	7.598	72.615	115.546	0.0017

Quinine was the most abundant alkaloid in polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon)**.**

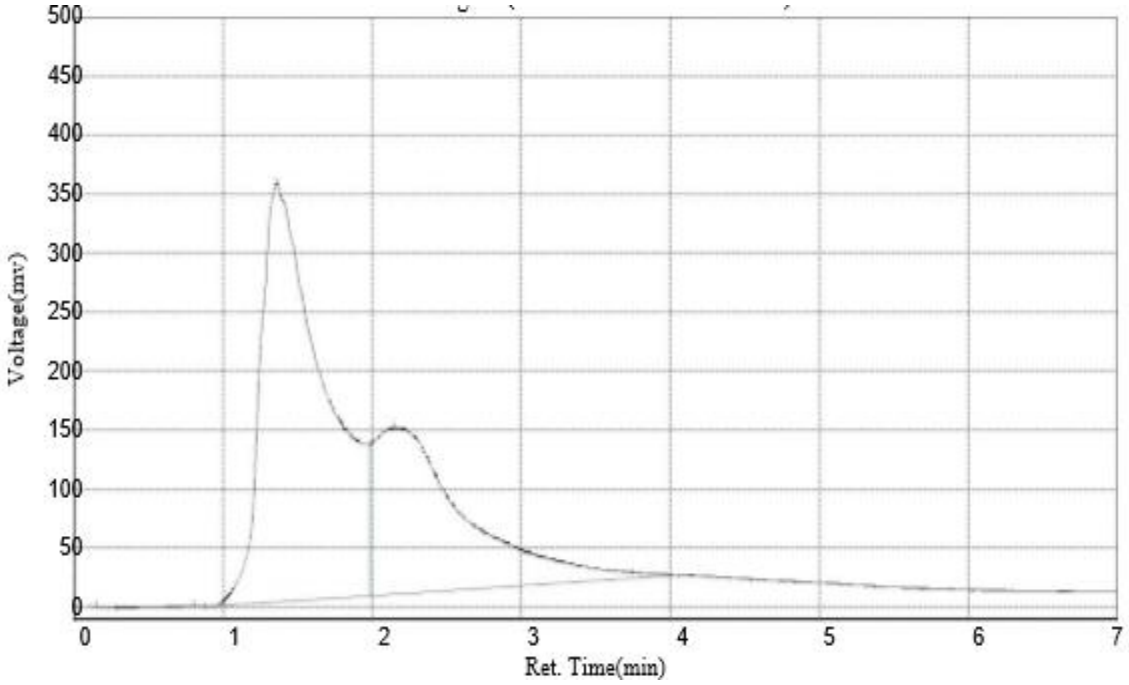


Figure 7: Chromatogram indicating steroids present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) as characterized with HPLC.

Table 6: Steroids in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) characterized with HPLC

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/10ml
1	Unidentified	0.140	293.857	1281.800	0.0077
2	Antheridol	0.798	283.901	6547.250	0.0394
3	Diosgenin	1.357	354365.781	10527073.000	63.3265
4	Ergocalciferol	2.040	141340.813	6088582.500	36.6264

Diosgenin and Ergocalciferol were the most abundant steroid in polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon)**.**

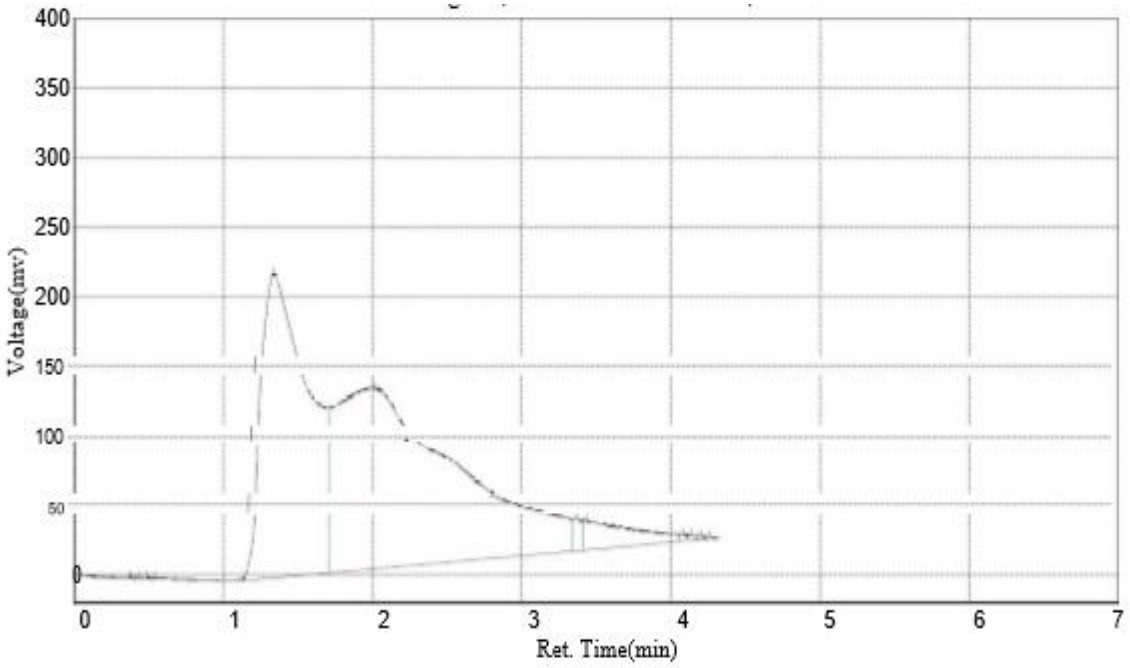


Figure 8: Chromatogram indicating tannins present in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) as characterized with HPLC.

Table 7: Tannins in polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) characterized with HPLC

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100ml
1	Unidentified	0.373	190.755	254.485	0.0020
2	Unidentified	0.432	369.898	757.207	0.0059
3	Unidentified	0.482	498.163	1263.807	0.0099
4	Unidentified	0.540	524.306	3223.852	0.0252
5	Epigallotechin	1.332	218465.000	4806506.000	37.6178
6	Catechin	2.007	130185.672	7360208.000	57.6042
7	Unidentified	3.365	23444.117	88777.891	0.6948
8	Unidentified	3.432	21357.973	479831.969	3.7554
9	Unidentified	4.082	4732.323	14621.866	0.1144
10	Unidentified	4.140	3645.072	10917.487	0.0854
11	Unidentified	4.198	2564.821	7248.313	0.0567
12	Unidentified	4.257	1490.570	3606.334	0.0282

Epigattotechin and Catechin were the most abundant tannins in polyherbal formulated tea (*Moringa olifera*, garlic, ginger, turmeric and lemon)**.**

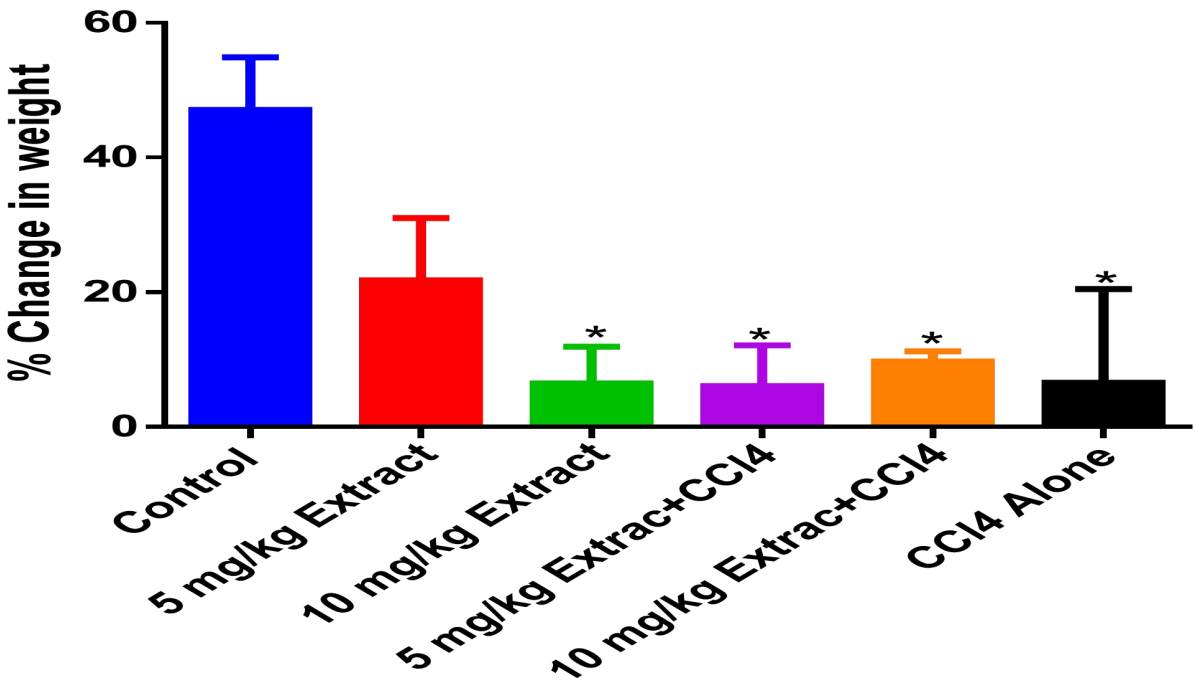


Figure 9: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on body weight on CCL4 induced hepatotoxicity in rat. There was weight reduction in the animals that were given 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 and CCL4 without treatment when compared to control (*$p<0.05$). Values were presented as mean±S.E.M, n= 4.

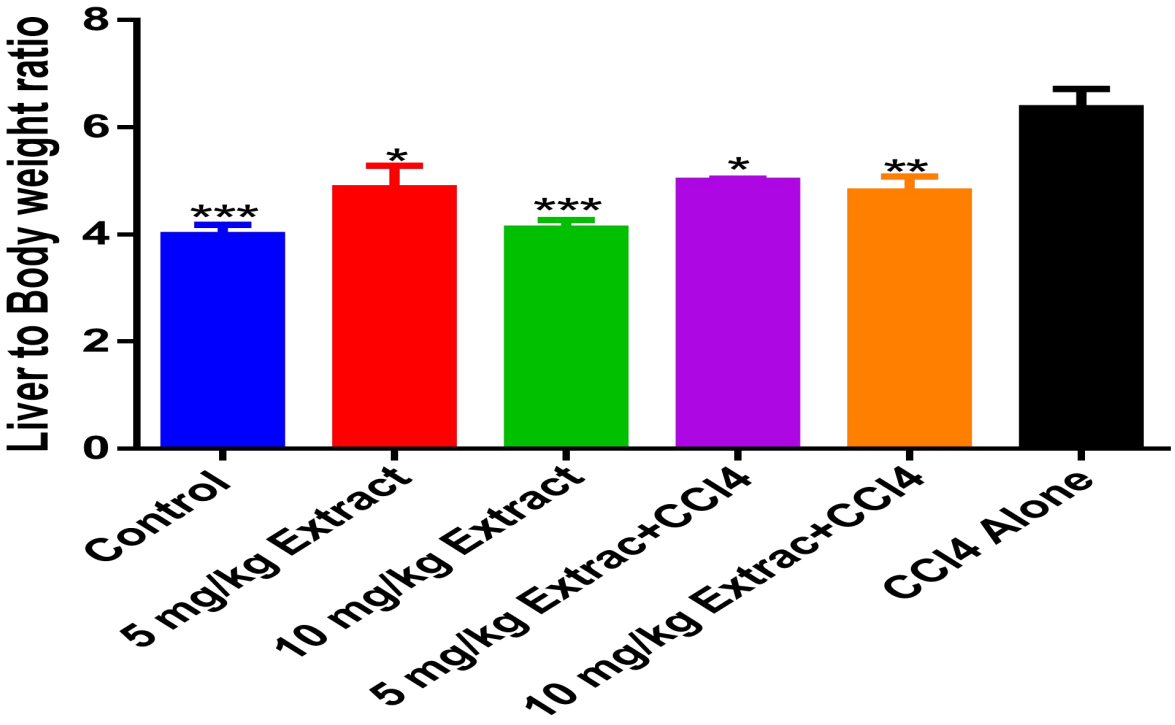


Figure 10: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on liver to body weight ratio on CCL4 induced hepatotoxicity in rat. There was liver weight reduction in the animals that were given distilled water, 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 when compared with CCL4 without treatment (***$p<0.001$; *$p<0.05$). Values were presented as mean±S.E.M, n= 4.

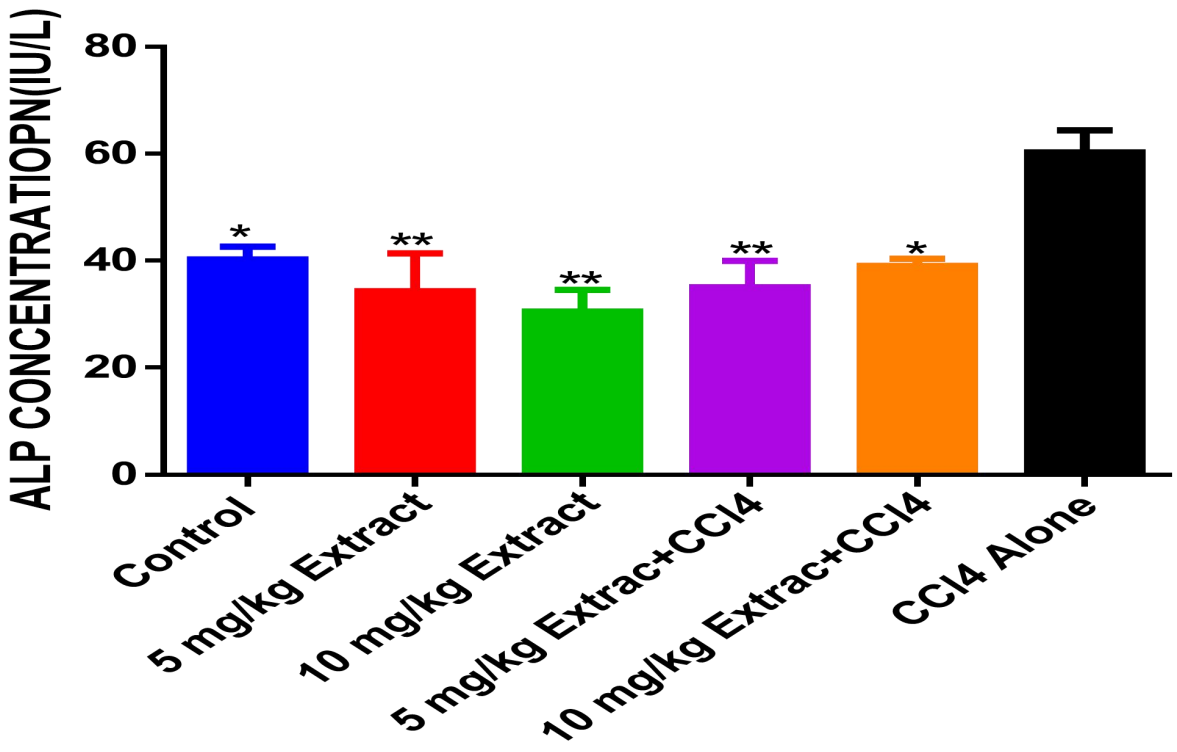


Figure 11: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on ALP on CCL4 induced hepatotoxicity in rat. ALP value reduce in the animals that were given distilled water, 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 when compared with CCL4 without treatment (**p<0.01; *p<0.05). Values were presented as mean±S.E.M, n= 4.

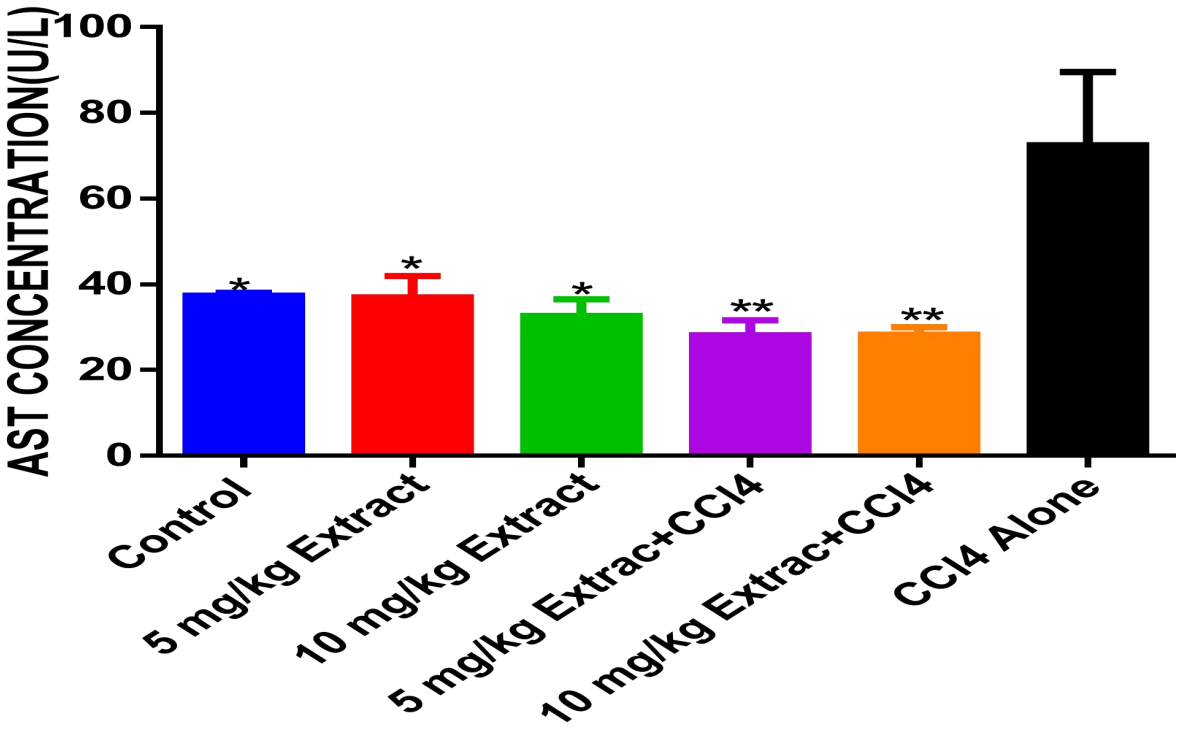


Figure 12: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on AST level in CCL4 induced hepatotoxicity in rat. AST value reduce in the animals that were given distilled water, 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4 and 10 mg/kg of extract + CCL4 when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$). Values were presented as mean±S.E.M, n= 4.

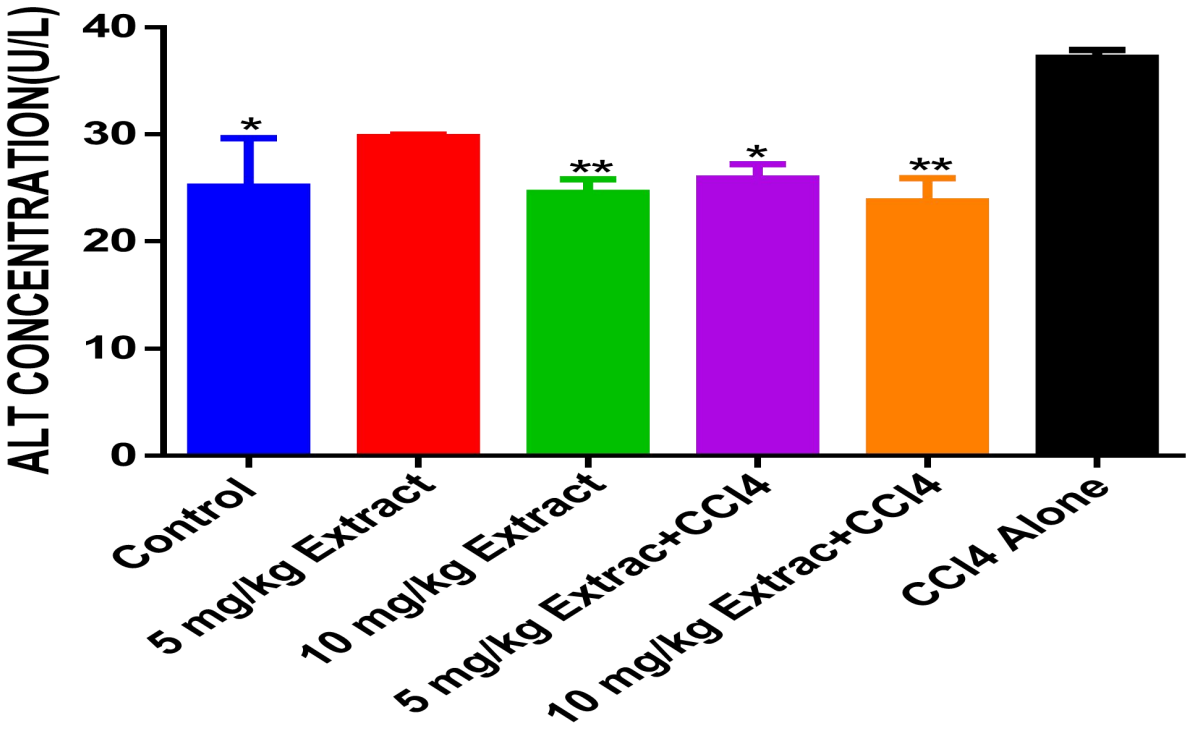


Figure 13: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on ALT level in CCL4 induced hepatotoxicity in rat. ALT value reduce in the animals that were given distilled water, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4 and 10 mg/kg of extract + CCL4 when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$). Values were presented as mean±S.E.M, n= 4.

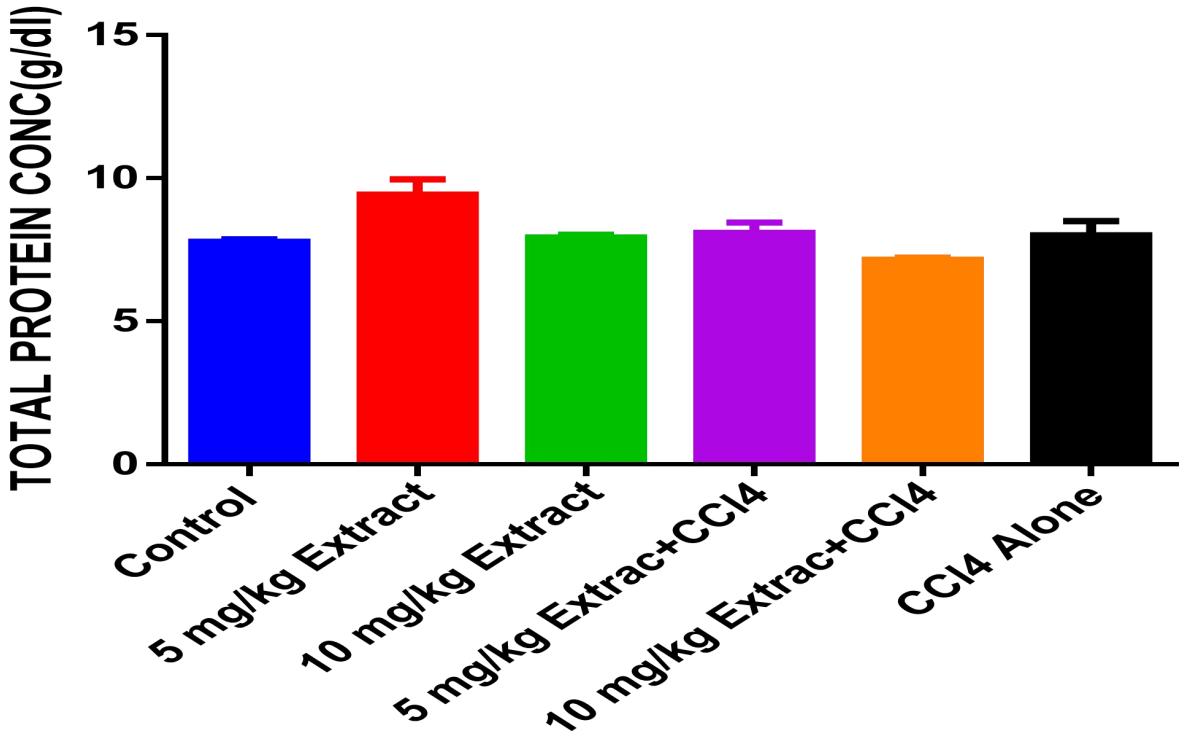


Figure 14: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on total protein in CCL4 induced hepatotoxicity in rat. There were no effect on total protein when compared with CCL4 without treatment (**p<0.01; *p<0.05). Values were presented as mean±S.E.M, n= 4.

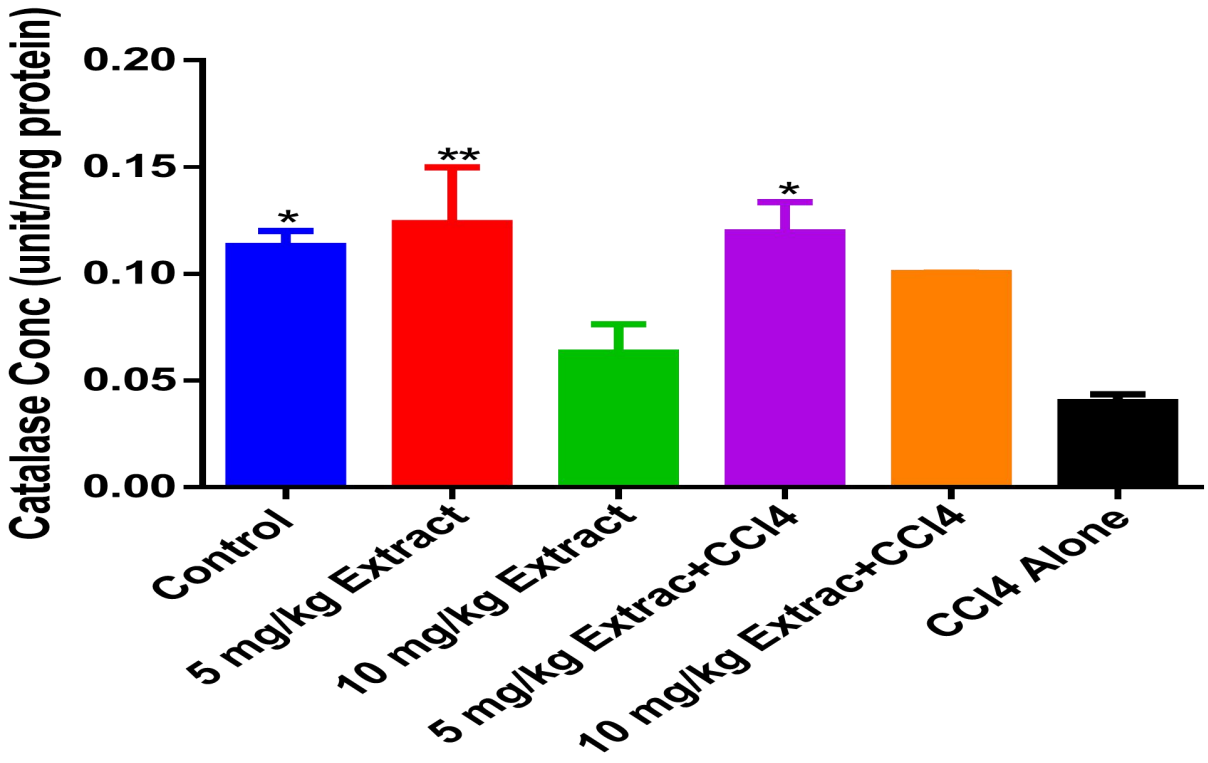


Figure 15: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on Catalase level in CCL4 induced hepatotoxicity in rat. Catalase value increased in the animals that were given distilled water, 5 mg/kg of the formulated extract and 5 mg/kg of the extract + CCL4 when compared with CCL4 without treatment (**$p<0.01$; *$p<0.05$). Values were presented as mean±S.E.M, n= 4.

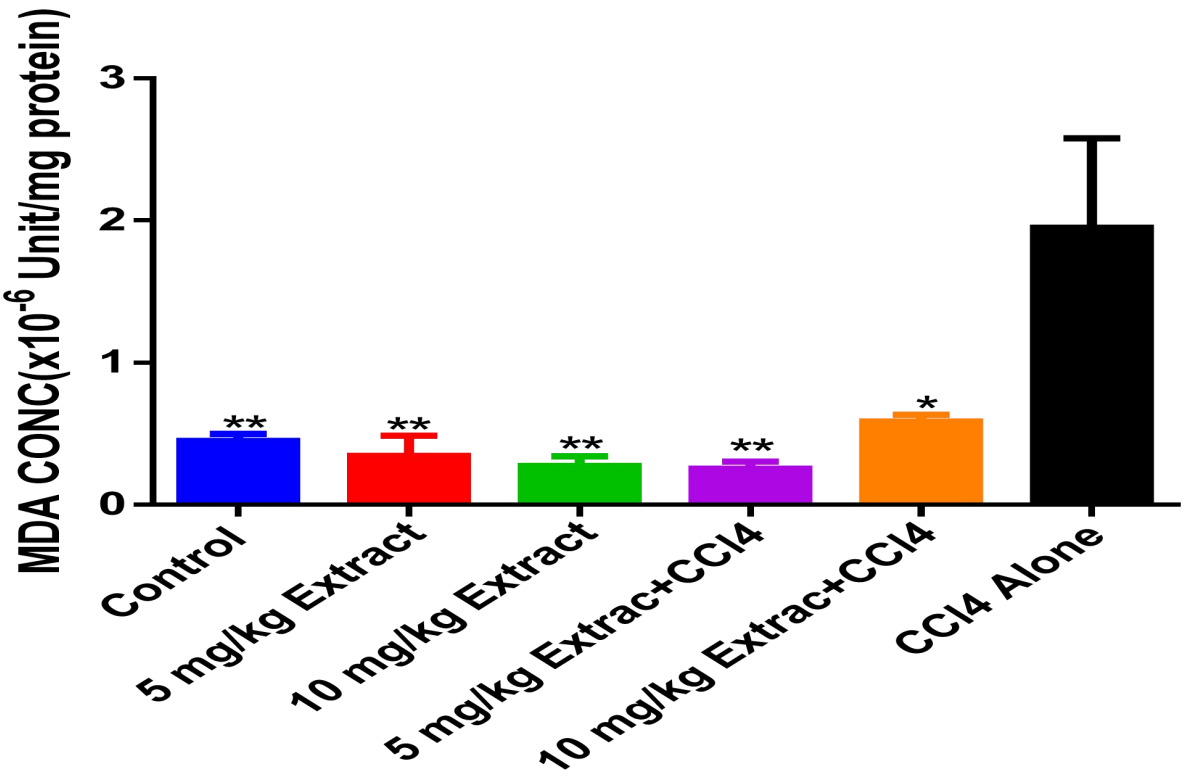


Figure 16: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on MDA level in CCL4 induced hepatotoxicity in rat. MDA value reduced in the animals that were given distilled water, 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4 and 10 mg/kg of extract + CCL4 when

compared with CCL4 without treatment (**p<0.01; *p<0.05). Values were presented as mean±S.E.M, n= 4.

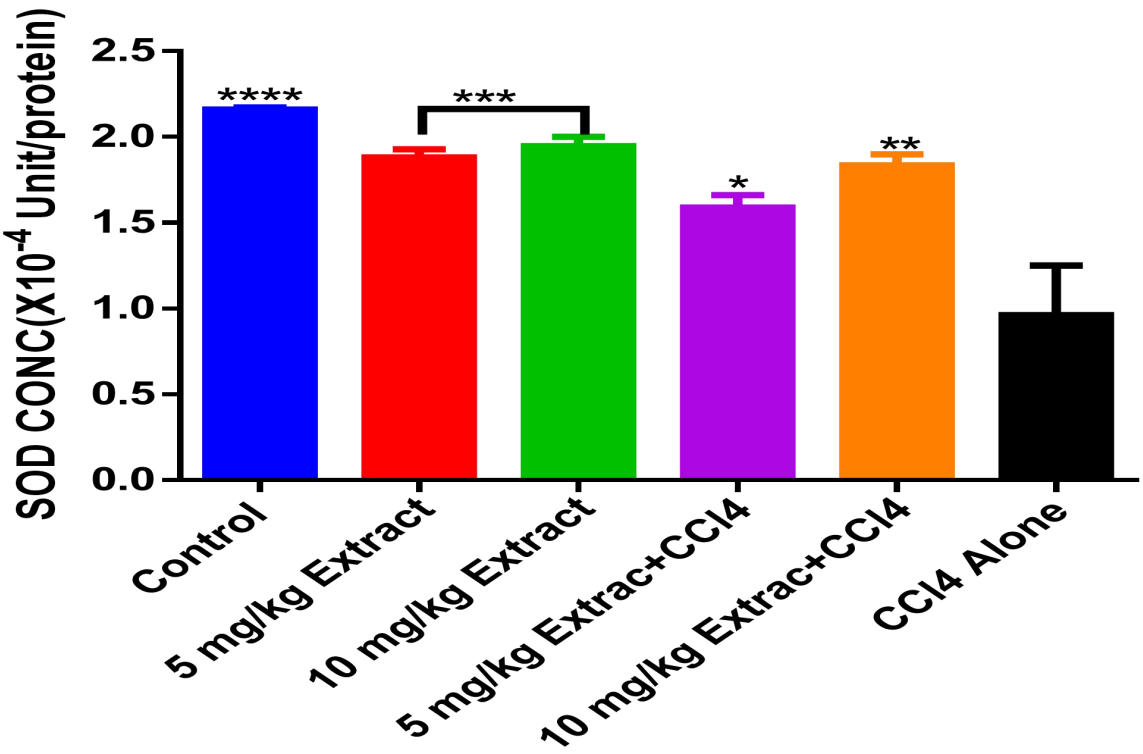


Figure 17: The effect of polyherbal tea formulation (*Moringa olifera*, garlic, ginger, turmeric and lemon) on SOD level in CCL4 induced hepatotoxicity in rat. SOD value increased in the animals that were given distilled water, 5 mg/kg of the formulated extract, 10 mg/kg of the

formulated extract, 5 mg/kg of the extract + CCL4, and 10 mg/kg of extract + CCL4 when compared with CCL4 without treatment (****$p<0.0001$; ***$p<0.001$; **$p<0.01$; *$p<0.05$). Values were presented as mean±S.E.M, n= 4.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

In India, more than ninety-three medicinal plants are used in different combinations in the preparation of forty patented herbal formulations (Vilas *et al*., 2011). However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy (Vilas *et al*., 2011). Polyherbals were found to have hepatoprotective activity due to their significant effect on liver enzymes, lipid profiles, and urea and creatinine levels (Saroj *et al*., 2012).In this study, the nutritional composition, phytochemical constituents, and hepatoprotective activity of poly-herbal tea (moringa olifera, turmeric, ginger, garlic, and lemon) in CCl_4-induced hepatotoxicity were evaluated. The nutritional and mineral composition of poly-herbal tea (moringa olifera, turmeric, ginger, garlic, and lemon) shows that it contains carbohydrates (64.66%) in a large amount when compared to other proximate content (Table 2), thus it can be used as a source of carbohydrates. They play important roles in the body as sources of energy as well as structural materials (Voet *et al*., 2008). The second largest proximate content present in poly-herbal tea is

protein (19.25%). Proteins, another class of food known as a "nitrogen-containing natural product," have been shown to be essential for human and animal survival (Voet *et al*., 2008), so it can also be a good source of proteins. Other minor components include fat (6.35%), moisture (6.12%), ash (0.24%), and fiber (3.36%). Ash ashing removes all of the organic material in the sample; ash contains inorganic plant components. Ash is also a sign of a plant's high digestibility (Ullah *et al*., 2013). The presence of fiber is important to human health because fibers are easily digested (Udayakumar and Begum, 2004). Dietary fiber is required for efficient digestion and waste removal. It has been shown to lower blood cholesterol as well as the risk of coronary heart disease, hypertension, constipation, diabetes, and breast cancer (Houghton, 2007). This shows that the polyherbal formulation is free from biological and physiological reactions, as seen in the content of moisture. This study shows that poly-herbal tea (*Moringa olifera*, turmeric, ginger, garlic, and lemon) contains minerals such as potassium (1356.0 mg/kg), calcium (821.3 mg/kg), magnesium (380.8 mg/kg), phosphorus (331.4 mg/kg), and iron (221.4 mg/kg) in large amounts when compared to otherelements (Table 3). These results agree with the fact that among macroelements in Nigerian agricultural products, potassium is the most abundant mineral (Oshodi *et al*., 1999). These elements support human biochemical processes by serving structural and functional roles as electrolytes (Nelson and Cox, 2008). Calcium is required for the maintenance of healthy bones, teeth, and blood (Hussainn *et al*., 2009; Igwenyi *et al*., 2014). The most well-known element in the biological system is iron. It has a diverse set of biological roles. In the metabolic process, iron plays a particular role. Iron's involvement in the body is firmly linked to hemoglobin and oxygen transmission from the lungs to tissue cells (Kruczek, 2005).

The medicinal properties of plants have been attributed to their constituents, with phytochemicals playing a major role (Schreiner *et al*., 2006). The phytochemicals are present in plants for their normal physiological activities and defense mechanisms, as well as contributing to their pigmentations, scents, and flavors (Saxena *et al*., 2013). In this study, the polyherbal tea formulation contains a large amount of phenolic compounds such as luteolin (30.58 mg/100g), arbutin (28.16 mg/100g), kaempferol (22.88 mg/100g), apigenin (10.63 mg/100g), and quercetin (7.60 mg/100g) when compared to others (Table 4). Quercetin has valuable hepatoprotective activities against multiple liver diseases as it has anti-inflammatory, antioxidative, and anti-apoptotic properties (Miltonprabu *et al*., 2017). Among plant metabolites, phenolics are reputed to play a noticeable protective role against several health disorders (Hun *et al*., 2006). Phenolics possess various biological activities, for instance, ,anti-ulcer, anti-inflammatory, antidiabetic, antioxidant, cytotoxic, and anti-tumor (Shai *et al*., 2014). It was found that phenolic, especially polyphenolic, compounds such as flavonoids are very efficient scavengers of free radicals (Halliwell, 1994; Sathis *et al*., 2018) because of their molecular structures, which include an aromatic ring with hydroxyl groups containing mobile hydrogen. Also, research has shown that phenols play a significant role in reducing oxidative stress (Presti *et al*., 2017; Mustafa *et al*., 2021). In this study, quinine (99.99 %) was the most abundant alkaloid in the polyherbal formulated tea (Table 5) **.** This indicates that polyherbal formulated tea can be very effective in the management of malaria. The most abundant steroids in polyherbal formulated tea were diosgenin and ergocalciferol (Table 6). Epigattotechin and catechin in were the most abundant tannins in polyherbal formulated tea (Table 7)**.** Studies have shown that tannins have an effective superoxide, DPPH, and ABTS radical scavenging activities, H2O2 scavenging activity, Fe3+ reducing power, and metal chelation on ferrous ion activities

(Gulcin *et al.*, 2010). Thus, this indicates that polyherbal tea formulation possess antioxidant activity and can be used for illnesses associated with oxidative stress.

An important requirement in toxicological experiments is the ability to assess the effects of xenobiotics on specific organs. Organ weight can be the most sensitive indicator of the effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailey *et al*., 2004). Thus, a 20% difference in body weight between animals should be accompanied by a 20% difference in the weight of each organ. This relationship is based on the assumption that the ratio of organ weight (Y) to body weight (X) within each treatment group is constant (i.e., Y/X =). Based on the study of Bailey *et al*. (2004), liver and thyroid gland weights are best compared using organ-to-body weight ratios, and adrenal gland and ovary weights are best compared using organ-to-brain weight ratios. In this study, the effect of polyherbal tea on body weight showed a significant weight reduction in the animals that were given 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, 10 mg/kg of extract + CCL4 and CCL4 without treatment when compared to the control (Figure 9). Also, the effect of polyherbal tea on the liver to body weight ratio on CCL4 induced hepatotoxicity in rats indicates a significant liver weight reduction in the animals that were given distilled water, 5 mg/kg of the formulated extract, 10 mg/kg of the formulated extract, 5 mg/kg of the extract + CCL4, and 10 mg/kg of the extract + CCL4 when compared with CCL4 without treatment (Figure 10). Bailey *et al*., (2004), in their studies, say that growth retardation such as dietary restriction and significant stress induced by treatment with experimental compounds will result in artifactual changes in the relative weights of some organs, unless there are specific toxic effects on those organs (Bailey *et al*., 2004). Liver weights may be influenced by dietary factors that induce hepatic enzymes

(Amacher et al., 1998). Thus, the reduction in weight caused by the polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric, and lemon) can be a result of the induction of hepatic enzymes.

It is well established that hepatotoxicity by CCl4 is due to the enzymatic activation of the released CCl3 radical in a free state, which in turn disrupts the structure and function of lipid and protein macromolecules in the membrane of the cell. (Achliya *et al*., 2004; Anil *et al*., 2011). When hepatocytes are damaged due to any cause, an increased level of enzymes, such as AST, ALT, ALP, etc., are released from damaged hepatocytes into the blood. Similarly, when hepatocytes are damaged by CCl4, it also raises the serum level of AST, ALT, ALP, and bilirubin (Alkreathy *et al*., 2004). Marked elevations in serum levels of ALP, AST, and especially ALT are specific indicators of liver injury (Anand *et al*., 2011). The effect of polyherbal tea formulation on liver function markers in this study shows a significant reduction in ALP, AST, and ALT (Figures 11, 12, and 13, respectively) in the animals that were given distilled water, 5 mg/kg of the formulated tea, 10 mg/kg of the formulated tea, 5 mg/kg of the formulated tea + CCL4, and 10 mg/kg of the formulated tea + CCL4 when compared with CCL4 without treatment. This indicates that a polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric, and lemon) can be a good hepatoprotective formulation in the management of liver injury. In addition to the hepatoprotective effect of polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric, and lemon), the results of this study show that polyherbal tea formulation significantly increased the levels of catalase (Figure 15) and superoxide dismutase (Figure 17), caused a reduction in the level of MDA (Figure 16), and had no effect on total protein (Figure 14) in animals given distilled water. Studies have shown that superoxide dismutase (SOD) plays a key role in fighting against oxidative stress-related

pathophysiologies, such as ischemia reperfusion injury, hypertension, and lung injury (Aziz *et al*., 2019). MDA, on the other hand, provides an index for the extent of perioxidative damage that has occurred in the liver (Ozolua *et al*., 2019). Thus, this study shows that a polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric, and lemon) can reduce liver injury through its antioxidant effect.

Conclusion and recommendation

The study showed that this polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric, and lemon) provides significant protection against liver injury caused by CCl4, which has potential for clinical application in the treatment of liver diseases. The current study also concludes that the polyherbal tea formulation (Moringa olifera, garlic, ginger, turmeric, and lemon) is hepatoprotective and contains minerals, nutrients, phytochemicals, and antioxidants. However, further study can be done to analyze the toxicity and other pharmacological effects of these polyherbal tea formulations.

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