HEPATOPROTECTIVE EFFECTS OF *AGERATUM CONYZOIDES* CRUDE EXTRACTS AND FRACTIONS ON CARBON TETRACHLORIDE–INDUCED HEPATOTOXICITY

By:

FREDERICK SARFO – ANTWI

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY

(BIOCHEMISTRY)

College of Science

DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

…….. ……..

Frederick Sarfo - Antwi Date

(PG4575515)

Certified by:

…… ……..

Dr. Christopher Larbie Date

(Supervisor)

Certified by:

…… ……..

Dr. Peter Twumasi Date

(Head of Department)

ABSTRACT

Liver diseases are still a major public health burden in many countries and despite the research strides made in respect of therapeutic interventions, challenges still abound in its treatment. Chemotherapy, the standard treatment method, is fraught with various shortcomings like drug resistance and undesirable side effects. Consequently, natural products present promising alternatives to liver disease treatment and hence finding new hepatoprotective agents from natural sources will be critical in improving patient outcomes. *Ageratum conyzoides*, an annual herb is increasingly being used in folklore for the treatment of a wide range of diseases including liver damage caused by free radicals. The study was aimed at investigating the hepatoprotective and *in-vivo* antioxidant enzyme activity of *A. conyzoides* crude extracts and its fractions, as well as evaluating the phytochemical constituents of 50% hydroethanolic extracts and fractions of *A. conyzoides*. Free radical scavenging activity of the extracts was investigated (Table 4.1). Hepatoprotective activity of *Ageratum conyzoides* plant extracts was studied by measuring the level of liver enzymes (Figure 4.11), as well as, *in-vivo* antioxidant enzyme activity after treatment with CCl_4 (Figure 4.18-20). The presence of tannins, flavonoids, alkaloids, terpenoids and saponins were qualitatively screened for in the hydroethanolic crude extract and its isolated fractions (Table 4.3). The results showed that the crude extracts scavenged DPPH free radical in a concentrated dependent manner compared to the positive control, ascorbic acid. Phenolic compounds were detected in the crude extracts (Table 4.1). The 50% hydroethanolic crude extract recorded the strongest scavenger of DPPH, with 50% effective concentration (EC_{50}) of 0.14±0.01 mg/ml. It also had the highest total phenol content, (0.39± 0.14 mg/g) with ethyl acetate fraction having the weakest antioxidant potential (0.29 ± 0.06 mg/ml) when compared to the standard (Table 4.2). Thus excluded from hepatoprotective study. In the hepatotoxicity study, crude extract

treatment resulted in significant increase ($p<0.001$) in SOD, CAT and GSH levels (Figure 4.18-20) and a significant decrease ($p<0.005$) in MDA and MPO level against CCl_4 both in liver and kidney (Figure 4.21-22), hence suggesting it to have the strongest hepatoprotective potential when compared to aqueous and methanol fractions. Also, histological findings (Figure 4.16-17) also indicated the absence of fibrosis and necrosis in both the kidney and liver of rat treated with the crude extracted after CCl_4 (1 ml/kg b.wt) intoxication, whereas other treatment groups showed mild inflammation and cellular structure distortion of organs. The present investigation suggests that *Ageratum conyzoides* crude extract possesses remarkable hepatoprotective and nephroprotective properties and the compounds such as phenols, flavonoid and alkaloid detected could be responsible for these properties.

ACKNOWLEDGMENT

I wish to express my sincerest thanks to my supervisor Dr. Christopher Larbie. This thesis would not have been complete without his expert advice and unfailing patience. I am also most grateful for his faith in this study especially in the sometimes-difficult circumstances in which it was written. I would like to express a special word of thanks to Dr. Regina Appiah-Oppong, Head of Clinical Pathology Department, Noguchi Memorial Institute for Medical Research, who offered me some reagents for this work. A special thanks to Dr. Babatunde Duduyemi who also helped in the histopathological examination. God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC	*AGERATUM CONYZOIDES*
ALP	ALKALINE PHOSPHATASE
ALT	ALANINE AMINOTRANSFERASE
AST	ASPARTATE AMINOTRANSFERASE
BHT	BUTYLATED HYDROXYL TOLUENE
CAT	CATALASE
CCl_4	CARBON TETRACHLORIDE
CYP450	CYTOCHROME P450
DPPH	2, 2 – DIPHENYL-1-PICRYL HYDRAZYL
DTNB	5,5′-DITHIOBIS (2-NITROBENZOIC ACID)
EC_{50}	EFFECTIVE CONCENTRATION AT 50%
EDTA	ETHYLENEDIAMINETETRAACETIC ACID
$FeCl_3$	IRON (III) CHLORIDE
FTIR	FOURIER TRANSFORM INFRARED
GAE	GALLIC ACID EQUIVALENT
GC-MS	GAS CHROMATOGRAPHY MASS SPECTRUM
GGT	GAMMA-GLUTAMYL TRANSFERASE
GSH	REDUCED GLUTAHIONINE
GSH-Px	GLUTATHIONE PEROXIDE
GST	GLUTATHIONE S-TRANSFERASE
H_2O_2	HYDROGEN PEROXIDE
MDA	MALONDIALDEHYDE

MPO	MYELOPEROXIDASE
ROS	REACTIVE OXYGEN SPECIES
PUFA	POLYUNSATURATED FATTY ACID
SEM	STANDARD ERROR MEAN
SD	SPRAGUE – DAWLEY
SOD	SUPEROXIDE DISMUTASE
TBA	THIOBARBITURIC ACID
TCA	TRICHLOROACETIC ACID
TGFβ	TRANSFORMING GROWTH FACTOR BETA
TNFα	TUMOR NECROSIS FACTOR ALPHA
WHO	WORLD HEALTH ORGANIZATION

CHAPTER ONE

1.0 INTRODUCTION

Liver, an important organ in the human body, it is responsible for metabolism and detoxification of carbohydrate, protein, fat and unwanted substance from the body (Opoku *et al.,* 2007). The liver is made up of different types of cell, including biliary epithelia cells, stellate cells, sinusoidal endothelial cells, hepatocytes and kupffer cells. The hepatocyte is responsible for majority of the metabolic and synthetic roles of the liver and also account for about 70% of the total liver cells (Si-Tayeb *et al.,* 2010). Hepatic cells are also involved in contrastive enzymatic metabolic activities, a damage to this organ is therefore likely to cause malfunction in the body metabolism (Porchezhian *and* Ansari 2005).

Hepatotoxicity is liver injury which usually occurs when the liver is pre-exposed to chemicals (Bahirwani and Reddy 2014). In experimental models, chemical toxins such as carbon tetrachloride (CCl_4), acetaminophen, thioacetamide and galactosamine are mostly used to study injury of the hepatocytes both *in vivo* and *in vitro* conditions (Kim *et al*., 2014). The presence of CCl_4 results in the generation of tricholoromethyl radicals which induce toxicity in rat liver (El-Sayed *et al.,* 2015), it also increases lipid peroxidation in the hepatic cells and eventually damage the liver (Weber *et al.*, 2003). High level of precursors for reactive oxygen species as well as decrease in inhibitory effectiveness and scavenging mechanism of the body can be one major factor causing oxidative stress in the body. Amplification of such stress can lead to tissue damage, cell death and depletion of scavenging system of the body. Dynamic and highly integrated cellular stress can result in damage of organs such liver (Friedman, 2000).

Due to the global rise of liver diseases, tremendous scientific advancement in the field of medicinal plants has been made in recent years (Palanivel *et al*., 2008).

Medicinal plants play a role in the health promotion of humans. Majority of people in the world depend on medicinal plants for treatment of various diseases (WHO, 2005). In the light of modern science, medicinal plants must be given detailed studies. About 7500 plants have been estimated to have medicinal purpose and used in local health treatment, mostly in rural and tribal villages. (Kumar *et al.,* 2014). Out of these, the real medicinal potency of about 4000 of these plants are still unknown or little known. It is therefore important that detailed investigations, pharmacological evaluation and documentation of these herbal plants are been implemented to ensure development of traditional medicine for many dreaded diseases (Boadu and Asase, 2017)

Ageratum conyzoides is a weed known to originate from American tropical regions and widely spread to numerous tropical and savannah regions of the world (Nogueira *et al*., 2010). It is a weed commonly found in most parts of Ghana and also has medicinal purpose including treatment of fever, pneumonia, cold, rheumatism, spasm, curing wounds and headache (Shirwaikar *et al.,* 2003). It has radical scavenging potential (Jagetia *et al*., 2003; Adebayo *et al*., 2010), hepatoprotective effects and can also serve as booster of blood (Ita *et al*., 2009). It had all been verified through pharmacological investigation.

Studies conducted by Kamboj and Saluja, (2008) reported phytochemicals such as alkaloids, resins, saponins, tannins, glycosides and flavonoids to be present in *A. conyzoides*. Glycoside is an example of bioactive principles that have also been identified and isolated in *A. conyzoides* (Nyunaï *et al*., 2010). The main purpose of this research was to determine the hepatoprotective activity of the 50% hydroethanolic

crude extracts of *A. conyzoides* and its fractions against carbon tetrachloride-induced hepatoxicity.

1.1 PROBLEM STATEMENT

In recent years, liver diseases have increased rapidly (Palanivel *et al.,* 2008). In addition, the development of liver diseases has also become a global issue and their treatment and management mostly result in more economic problems, especially in African. Many side effects are also related with synthetic drugs used in the treatment of hepatic disorders. There is therefore the need to explore other natural products of plant origin for their hepatoprotective effect and further evaluate their modulatory effect on endogenous liver antioxidant enzymes.

1.2 STUDY OBJECTIVES

This study aimed at determining the hepatoprotective activities of 50% hydroethanolic leaves extracts of the *Ageratum conyzoides* and its fractions on carbon tetrachloride - induced hepatotoxicity.

1.2.1 Specific Objectives

This work specifically sought to:

a) Determine the hepatoprotective and *in-vivo* antioxidant enzyme activity of *A. conyzoides* crude extracts and its fractions.

b) Evaluate qualitative phytochemical constituents of the crude extract and its

fractions.

c) Determine the *in vitro* antioxidant activity of the crude extract and its fractions.

1.3 JUSTIFICATION

Liver diseases remains a major health concern nationwide, thus resulting in increased morbidity and mortality all over the world, with available treatment options being very costly. Due to this, majority of people, especially African countries including Ghana seek traditional herbal medicine as treatment option for liver and other diseases. Many active extracts of plants are mostly used to treat different variety of clinical diseases including liver diseases (Iwu *et al.*, 1999). Most traditional medicines do not have a defined dose and potency data, however they may contain many specific bioactive compounds in their natural state, which can influence the human biological process (Strobel and Daisey 2003). Therefore, the search for effective and safe medicine for liver disease treatment continues to be an area of focus.

Antioxidants enzymes scavenge free radicals and protect the cells against the tremendous effects by these unstable substances. Examples of these unstable free radicals include singlet oxygen, peroxyl radicals, hydroxyl radicals and peroxynitrite anion. ROS is responsible for initiating a series of processes that can result in DNA damage in some cell such as liver, which can gradually lead to dysplastic cellular appearance and dysregulated growth. The formation of polyunsaturated fatty chain reactions of free radicals is prevented by antioxidants sometimes found in food supplements (Doughari, 2012). Many industries especially pharmaceutical companies have attention now on natural antioxidants to replace synthetic compound mostly located in their products due to safety concern of these synthetic compounds

(Doughari, 2012). Consumers' preferences for natural antioxidants products have increased in the current market, thus giving source of antioxidants more impetus to explore (Granato *et al.*, 2010).

Traditional herbal plants have been used for various diseases treatment for several years, due to their low toxicity, thus resulting in a lower side effects. Many drugs currently available were derived from medicinal plant either directly or indirectly. Global interest in natural therapies and alternative medicines has made scientists and researchers to pay much attention to traditional medicine, especially in Africa. Hence, it has become necessary to presently investigate *A. conyzoides* for its hepatoprotective, antioxidant and phytochemical constituents.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 LIVER

The liver is an essential organ that help sustain human life. It's found in the right upper region of the abdomen, mostly behind the rib cage, and performs crucial functions including metabolism of drugs and toxin, remove degraded products of cellular metabolism including ammonia and bilirubin, and also involved in the synthesis of many important proteins and enzymes. The liver is made up of two parts, namely the right and left lobe. Falciform ligament joins the coronary ligament at the liver's superior margin. It also mark the external division on the front of liver (Stringer, 2014). The liver serves as a chemical processing plant in the body which receive about 30 % of the resting cardiac output. Nutrients and other important substances are stored in the liver, as well as degradation of toxic substances. Toxins such as bile and urine are excreted out from the body through the liver and kidney respectively. The liver plays a key role in detoxification of substances such as xenobiotics, chemotherapeutic agents and environment pollutants, thus making it more susceptible to variety of liver disorders caused by either these agents or their metabolites. Liver disease limits its ability to perform its life preserving functions (Edmonds and Francesconi, 1993).

2.2 EPIDEMIOLOGY OF LIVER DISEASES

When the liver become damaged beyond repair, it's unable to perform its routine function. Urgent medical care is always needed anytime the liver becomes damaged. Damage usually occurs gradually and over many years results into liver disease, however, acute liver disease which rarely occurs may be difficult to detect at the early

stage. Acute liver failure, a clinical syndrome that mostly occurs due to massive liver necrosis can leads to the development of rapid hepatic encephalopathy and cause severe impairment, thus resulting in hepatic malfunction (Garcia-Tsao and Lim, 2009). Mostly, in acute liver failure, a normal healthy liver suffers tremendous damage that results in cellular changes and symptoms of the liver. Most common cause of acute liver failure include the ingestion of poisonous toxin such as wild mushrooms, reactions to certain prescription and herbal medication, or overdose of certain drugs such as acetaminophen (Gill and Sterling, 2001). Development of encephalopathy in acute liver failure can result in the accumulation of toxic substances in the body due to the liver's inability to metabolize it. Patients with acute liver diseases are likely to die within few days after the onset of symptoms if immediate treatment is not taken. Patients with chronic liver disease can survive in the absence of early treatment.

The liver can repair itself when affected. Continuous exposure to injury results in a permanent damage to the liver, resulting in scarring also termed as cirrhosis.

2.3 CAUSES OF LIVER DISEASES

There are numerous ways the liver can be damaged and prevented from performing its normal functions. Though there are still unknown causes for many liver diseases, the most frequent liver diseases are mostly caused by one of the following factors:

2.3.1 Cancer and growth

The liver can be affected by primary liver cancer, which mostly arise from the liver or cancer formed in other parts of the body and gradually spreads to the liver through metastasis. Liver cancer usually start elsewhere in the body. About 2 % of liver disease

in United State is because of cancer (Bosch *et al.,* 2004). The prevalence of hepatitis caused by contagious viruses sometimes predispose a person to liver cancer. The availability of many different cells of the liver makes it possible for several types of tumours formation. Examples of liver cancer include Hepatic adenoma and cholangiocarcinoma. Cirrhosis is also common with patient with liver cancer.

2.3.2 Virus infection

Primary attack of viruses on the liver cells can lead to inflammation of the liver, representing the commonest causes of the liver injury worldwide. In the United State, viral hepatitis account for the death of more than 15,000 people annually (Wasley *et al.*, 2008). Common viruses mostly responsible for infection of the liver include Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D and Hepatitis E. These viruses result in acute diseases with symptoms such as abdominal pains, fatigue, nausea, malaise and jaundice, however chronically infected patients can develop cirrhosis and hepatocellular carcinoma (Wasley *et al.*, 2008). In addition to the numerous hepatitis viruses available, adenovirus, cytomegalovirus, epstein –barr virus and yellow fever are other viruses that can cause inflammation of the liver. In 1997, there were reported cases of viral hepatitis caused by Herpes simplex virus (Kaufman *et al.*, 1997).

2.3.3 Immune system abnormality

Autoimmune hepatitis is a chronic disease which occurs when there is an abnormality in the immune system, thus the body's immune system turns to attack its normal components such as liver cells and eventually cause inflammation and liver damage. The role of the immune system is to help the body in fighting against bacteria, viruses

and other invading organisms, however researchers have not been able to prove why the body sometimes attacks itself in such manner, although heredity and other infections may play a key role (Murphy and Weaver, 2016).

2.3.4 Excessive exposure to free radicals

Free radicals are described as unstable and highly reactive molecules. These free radicals could be generated by the body through oxidative stress or introduced to the body through drugs such as acetaminophen which has a high probability of producing unstable radicals when taken in overdosed. Free radical generation and elimination is very important because excessive generation of cellular radical can be harmful and cause damage to cells especially the liver (Rice-Evans *et al.*, 1997). Free radicals destroy the liver cell membrane through the formation of a long polyunsaturated fatty acid chain precursors through radical reaction of intermediate compounds involving oxygen and metal cations. Inflammation of the liver usually result from these combined reactions (Nishigaki *et al.*, 1981).

2.4 LIVER DISEASE AS A PUBLIC HEALTH BURDEN

In China, about 300 million people are affected by liver diseases, thus a global impact on the health. According to the World Health Organization (WHO, 2009), 46% global diseases recorded are liver disease and about 35 million people worldwide die of such diseases (Murray and Lopez, 1996). Diseases of the liver had increased rapidly over the years. Liver diseases have been ranked as one of the diseases that cause frequent deaths according to the National statistics in the United Kingdom. About 4.5 % to 9.5 % of autopsy in worldwide studies reported deaths to be due to liver diseases. Hence,

we can estimate that majority of people especially the working population would be affected with such diseases. During 2001, 771,000 people were reported to have died from liver disease (Lim and Kim, 2008).

About 75% of patients with liver diseases experience progressiveness of the diseases despite the therapeutic treatment given and eventually die within one to five years from serious complications (Wood *et al.* 2000). In the natural history of liver disease, it has been reported that about 10% to 33% of people who develop such disease end up with serious complication (Lin *et al*., 2008). Liver disease is considered as a public health problem that necessarily need high priority strategies for its prevention and control (Lin *et al*., 2008). Studies conducted on hepatic diseases by Edington in Ghana showed that cirrhosis was the most common liver disease leading to the death of many citizens in the country (Edington, 1957). In Ghana, data on the prevalence and death rate from the period 1980 to 2000 obtained in the Department of Pathology, Korle Bu Teaching Hospital (KBTH) confirmed this observation (Blankson *et al*., 2005). Disease of the liver is considered among the commonest disease in Ghana causing death however, the systematic investigation on the relative causes and other possible risk factors have not yet been extensively dealt with. The government through the Ghana Health Service should embark on immediate actions to help reduce the rate of liver diseases in the country. Treatment method are mostly costly, thus making it difficult for others to patronize. Government should seriously consider including some treatment options that are inexpensive and most effective and efficient. Besides, the Ghana Health Service should also conduct extensive research on liver disease to help promote the awareness of such diseases.

A study conducted in a hospital in Accra, Ghana, between 1996 and 2002 has shown that out of 22,394 persons who died and passed through autopsies, 1,176 (5.25%)

suffered from liver diseases. The study also revealed that liver diseases had been rising at a rate of about 0.3 percent annually in Ghana with more men suffering and dying from liver diseases than women. In addition, tests conducted during blood donations indicated that cases of Hepatitis was increasing in the country (Dongdem *et al.*, 2012).

Drugs and chemicals with associated hepatotoxicity effects are most employed as inducers for various hepatotoxicity testing. Some analgesics like paracetamol, aspirin and phenylbutazone have also been documented to cause liver damage (Manov *et al*., 2006). Commonly used tuberculosis drugs such as isoniazid could also result in liver damage; in about 20 patients, 1-2 patients experience severe hepatotoxicity (Sarich *et al.*, 1999).

Fungi such as *Aspergillus flavus* and *Aspergillus parasiticus,* produce toxins which have been observed to have tremendous effects on the liver (Kanbur *et al*., 2011). Other chemical agents with negative effects on the liver include vinyl chloride, arsenic and carbon tetrachloride. There is therefore the growing need to research into liver diseases and hepatoprotective remedies especially from natural sources (Orhan *et al*., 2007).

2.5 EXPERIMENTAL MODELS OF HEPATOTOXICITY

2.5.1 Carbon Tetrachloride-Induced Hepatotoxicity

Carbon tetrachloride (CCl_4), is a very active chemical capable of causing injury to hepatocytes. The use of CCl_4 by many investigators to cause liver injury in rat had been successful. CYP2E1 enzyme from the Cytochrome P450 family located within the endoplasmic reticulum metabolize CCl_4 which results in the formation of a highly reactive free radicals (trichloromethyl). Formation of these free radicals can lead to the

activation of lipid peroxidation and protein oxidation, thus damaging the hepatocellular membrane (Gyamfi *et al.*, 1999). During metabolism of CCl_4, the trichloromethyl radical formed is capable of binding to lipids which could cause lipid peroxidation and damage the liver (Ahmed *et al*., 2000). Within 24 hours after administering a carbon tetrachloride to a rat, changes of centrilobular necrosis and fat occur, damaging the membrane and resulting in leakage of hepatic enzymes into the serum (Parola *et al*., 1992). CYP2E1 is the main enzyme responsible for metabolic activation of CCl_4 as indicated by the absence of toxicity in CYP2E1 knockout mice, thus dysfunction of CYP2E1 can avoid hepatic injury from carbon tetrachloride.

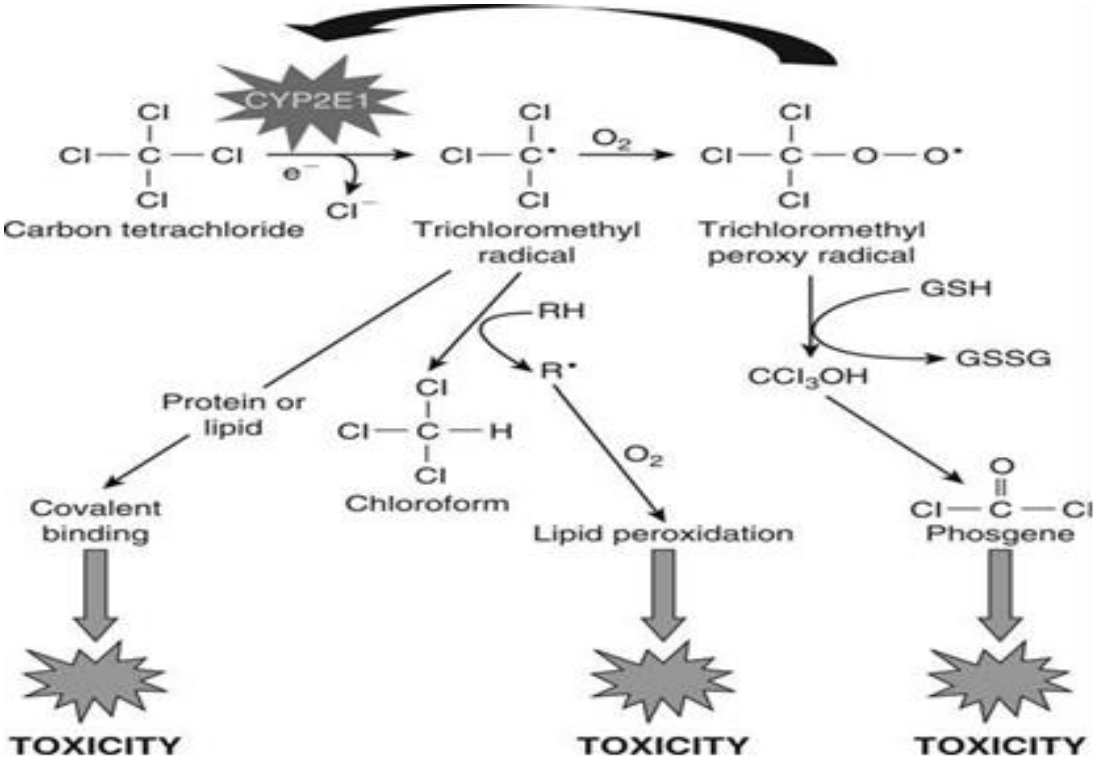

Figure 2.1: Mechanism of hepatotoxicity of CCl_4

(Adopted from Timbrell, 2009)

2.5.2 Paracetamol-Induced Hepatotoxicity

Drugs such as paracetamol cause liver injury when exposed to the body in high doses (Bhanwra *et al.*, 2000) but remain safe and effective when used at the therapeutic level (Rumack, 2004). Necrosis of the centrilobular hepatocytes occurs after paracetamol administration. Covalent binding of sulphydryl group to paracetamol (N-acetyl-P-benzoquinoneimine) leads in the breakdown and lipid peroxidation of glutathione level and hence causes damage in the liver cells (Bhanwra *et al*., 2000; Maheswari *et al*., 2008).

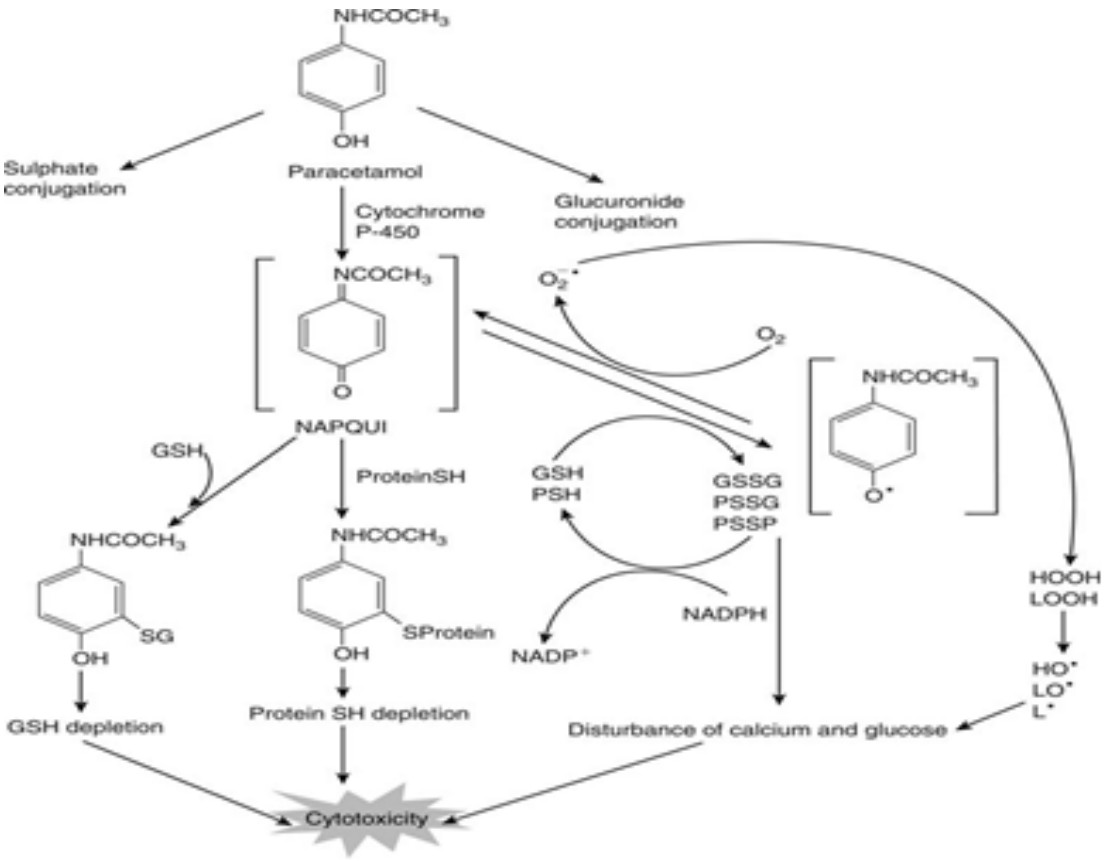

Figure 2.2: Metabolic activation of paracetamol.

(Adopted from Timbrell, 2009)

2.5.3 Metabolism of Drugs in liver

Metabolism of drugs usually take place in the liver. Typically, metabolism inactivate drugs, however some drug metabolites activate the drug pharmacologically. Methods of metabolizing drugs include oxidation, hydrolysis, hydration, conjugation, condensation or isomerization. Metabolism of enzymes usually occur in many tissues, however most of these enzymes are concentrated in the liver. Drugs metabolism varies from individual, these may be influenced by many factors, both genetic and environmental factors. Drug metabolism usually occurs in two stages, namely stage 1 and stage 2.

In stage 1, reaction involves the modification of functional group or cleavage through oxidation, reduction and hydrolysis mechanisms. In stage 2 reaction, conjugated endogenous substance formed are eliminated from the body. Chemically active and potentially toxic metabolites are mostly generated during the process of drug metabolism more especially in the liver (Liston *et al.*, 2001). Cytochrome P-450 is a group of enzymes which metabolize drugs in the liver.

2.5.4 Role of cytochrome p450 enzymes

Cytochrome P450 is made of superfamily of haem enzymes found in bacteria and humans (Nelson *et al.*, 1996). This type of enzyme is found in most plants and animal species. Cytochrome P450 is actively involved in oxidative metabolism function of many available drugs from a diverse number of drug classes. Examples include thiazolidinediones, meglitinides, antimalarial and chemotherapeutic taxanes. Cytochrome P450 has been studied more extensively (Daily and Aquilante, 2009). Liver, kidney, lung, intestine and brain are common catalytic oxidative reaction site of P450 isoenzyme (Yoshizawa *et al.*, 1998). They evolved to enable organisms to metabolize xenobiotics, a category that includes exogenous medications as well as

prostaglandins and endogenous hormones that resemble foreign substances (Sluzewska *et al.*, 1996). Many medication are metabolized by more than one P450 isoenzyme due to substrate overlap, thus may inhibit or activate more than one isoenzyme.

2.5.5 Drug induced liver injury

The liver's central role involves clearing and transforming of certain induced substance or toxins that is likely to cause damage to the liver. Drug that's induced liver injury are classified based on animal model predictability and dose-dependency. Liver diseases caused by drug-inducement may be predictable or unpredictable. Immune mediated hypersensitivity or non-immune reaction are mostly observed in unpredictable reaction, also called idiosyncratic (Stellon *et al.*, 1985). Potent predictable hepatotoxin of drugs are mostly recognised during clinical or testing phase of drug development. Chemicals and toxins with pharmacological hepatotoxicity property have predictable dose-response curve and in most case result in direct liver damage. For instance, overdose of paracetamol could cause injury shortly after the threshold for toxicity is reached (Pirmohamed *et al.*, 1998).

2.5.6 Mechanism of liver damage

Several mechanisms can result in the damage or injury of the liver. Intracellular organelle such as mitochondria which produces energy can be damage by many chemicals. Improper function of the mitochondria can cause hepatic cells to injure. CYP 2E1, a common enzyme in the cytochrome P-450 system can cause oxidative stress when activated (Jaeschke *et al.*, 2003). Accumulation of bile acid in the liver

could result from damaged hepatocyte and bile adduct cells, thus promoting tremendous damage to the liver (Patel *et al.,* 1998). Kupffer cells, fat-storing stellate cells and leukocytes are all non-parenchymal cells that function in the mechanism that result in the formation of liver damage. The enzyme CYP in most cases activate many hepatotoxic and including carbon tetrachloride, nitrosamines and polycyclic aromatic hydrocarbon from their inactive state to their active state responsible for liver injuries especially in experimental animals and humans. The activation of kupffer cells and inflammation of other cell, reactive oxygen species formation and cytokines such as TNFα and TGFβ all key mediators that enhance the damage of liver cells (Targhetta *et al*., 2008).

2.5.7 Patterns of injury

Hepatitis is the term used to describe the inflammatory disease of the liver, which may be acute or chronic. Acute hepatitis usually results from viral infection particularly hepatitis A, B and C viruses, however alcohol and paracetamol could also cause hepatic injury. Development of chronic liver disease could result from infection of hepatitis B and C virus which eventually results in fibrosis and cirrhosis of the liver (Gutierrez-Reyes, 2007).

2.5.8 Plants and Possible Hepatoprotection

The present of chemical constituents in some plants make them potential agents for liver protection (Bhawna and Kumar, 2009). Glycyrrhizin usually administered intravenously, is a popular hepatoprotective drug known to prevent development of hepatocellular carcinoma (Yoshida *et al*., 2007). Liver disease also rely on a number

of plants and traditional formulations for treatments (Schuppan *et al*., 1999). *In vivo* evaluation of aqueous extract of *Artemisia absinthium* has been used for liver disease treatment and was experimentally done using mice whose liver was immunologically induced by endotoxin (Amat *et al.*, 2010).

2.6 HEPATOPROTECTIVE EFFECT OF MEDICINAL PLANTS

In Ghana, numerous herbal plant medicines are available for diseases treatments, with focus on their hepatoprotective effects particularly against carbon tetrachloride agents. Example of medicinal plants include *Allium hirtifolium, Apium graveolen, Cynara scolymus, Berberis vulgaris, Calendula officinalis, Nigella sativa, Taraxacum officinalis, Prangos ferulacea, Allium sativum, Marrubium vulgare, Ammi majus, Citrullus lanatus, Agrimonia eupatoria* and *Prunus armeniaca* (Asadi-Samani *et al.*, 2015).

2.6.1 Anticancer properties of medicinal plants

In the United State, one major cause of death is cancer (Jemal *et al.*, 2005), of which one in four deaths recorded is as a result of cancer diseases (Shoeb, 2006). The rate of mortality caused by cancer has increased since 1990 (Parkin *et al.*, 2000). In the last five decades, treatment of cancer has focused on the usage of traditional herbal plants. *Ageratum conyzoides* is a medicinal plant reported to have anticancer properties, with the *in-vitro* activity evaluated and published (Acheampong *et al.*, 2015). Phytochemicals such as flavonoids, diterpenoids, triterpenoids and alkaloids present in plant are reported to enhance its anticancer properties (Lin *et al.*, 2008). More than 300,000 higher plants exist on the surface of the planet; therefore active research into

medicinal plants will help isolate more bioactive principles that can inhibit the growth of cancer cells and also elucidate its molecular mechanisms of action.

2.6.2 Medicinal Plants with Antioxidant Properties

Generation of ROS occur in the body during normal metabolic functions, however other highly reactive compounds can be introduced to the body from the environment and dietary xenobiotics (Bandyopadhyay *et al*., 1999). These molecules are highly reactive and possess unpaired electrons. The immune system antioxidants gene expression can be induced by activities such as environmental pollution, radiation, chemicals, toxins, fatty foods as well as free radicals generated by physical stress, thus all these activities can result in abnormal formation of antioxidant proteins in the body (Bandyopadhyay *et al*., 1999).

Electrically charged molecules which have unpaired electrons and can attack free electrons from other substance in order to stabilize themselves are referred as free radicals (Bandyopadhyay *et al*., 1999). Initial neutralization of free radicals may not completely destabilize its chain formation until subsequent free radicals are also deactivated. Oxidative damage has been noted as a major factor in molecular mechanisms of many diseases such as liver disease, in due of this, antioxidants capacity has been an essential feature of modern medications which can act on diverse level of the process to eradicate and control disease. Synthetic antioxidants, commercially available in plants, exhibit strong antioxidant effects (Hancock *et al*., 2001). This situation has motivated researcher on the need to investigate on plants with naturally occurring antioxidants.

2.7 AGERATUM CONYZOIDES LINNAEUS

Ageratum conyzoides, an herbal plant, belongs to the Asteraceae family. It's described as an invasive weed in many regions and commonly known as goat weed. In Nigeria, especially among the Yoruba natives of south-western Nigeria, it is referred as 'imiesu' (Dalziel, 1937), in Brazil, it is referred as 'catingo do bode' (Dalziel, 1937), while in Ghana it is popularly known as adwowakuro (Akan-Akyem), efoe momoe (Fanti), and gu-akuro (Twi) (Dalziel, 1937).

The plant can reach a maximum height of 1500 mm when located in an environment of favourable conditions. The leaves and stem surface of *A. conyzoides* are observed to be hairy. The leaves also have an odour reminiscent to that of the male goat. Axillary part of the plant bears 4-8 flowers heads which are arranged in a flat-topped and showy manner. The colours of the flower include white, blue or violet, three rows of oblong bracts surround the head of flower with the flowers protruding above the bracts. (Acheampong *et al*., 2015)

Figure 2.3: *A. conyzoide* whole plant of family Asteraceae

2.7.1 Geographical Distribution of Ageratum conyzoides

Ageratum conyzoides is described as annual herb found mostly in tropical regions. It is a weedy plant located in island habitats such pastures, waste land and roadsides (Swarbrick, 1997). Environment rich with mineral soils and high humidity is a good propagating condition for *A. conyzoides*. Soil with poor nutrient levels are usually unable to sustain the survival for such plants, thus occur rarely on area such as island which has poor soil fertility. Report from studies recorded the abundance of *A. conyzoides* plants at a distance closer to road (Chuihua *et al.,* 1998). It is present on the African continent and can be found in countries like Ghana, Nigeria and Gambia. It can also be found in South America, specifically in Brazil.

2.7.2 Pharmacological Properties of Ageratum conyzoides

The indigenous style of managing or treating specific disease conditions from the native's perspective which may reflect the living conditions of people in a given environment is said to be ethno-medicine (Landy, 1977). The concept of ethno-medicine is proven useful in studying indigenous therapeutic agents particularly during drug discovery or development. This has enabled researchers to understand fairly and suggest possible scientific explanations to the basis of most native treatment modalities (Neuwinger, 2000). *A. conyzoides* was reported by Marques *et al.* (1988) to have analgesic effect in clinic trials with patients with arthrosis. Study conducted by Leal *et al.,* (2000) also confirmed the plant's ability to reduce inflammation in damaged cells. *A. conyzoide* has been widely used as traditional herb plant in West Africa. The plant extract is also found to have antioxidant and insecticidal activities (Nour *et al*., 2010; Singh *et al*., 2013).

2.7.3 Bioactive Principles isolated from Ageratum conyzoides

Many bioactive compounds present in this plant has been reported to play a key role in its diverse biological properties. The bioactive principles mostly isolated were from the essential oil of the plant. These include flavonoids, alkaloids, triterpenes and sterols, monoterpenes and sesquiterpenes, coumarin, benzofuran, chromone and chromene (Bandaranayake, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 REAGENTS

All chemicals for antioxidant studies were purchased from Sigma-Aldrich, Germany. Chemicals for biochemical analysis were also purchased from Fortress Diagnostics, UK. All experiments were conducted at the Department of Biochemistry and Biotechnology, KNUST, Central Laboratory, KNUST, Department of Anatomy, SMS, KNUST.

3.2 PLANT MATERIAL

A. conyzoides leaves were handpicked from the land around the Atwea Mountain, Ashanti region, Ghana. The plant samples were authenticated based on a voucher specimen from Acheampong *et al.* (2015) study (KNUST/HMI/2014/WP005) deposited at the herbarium of the Department of Pharmacognosy, KNUST, Kumasi. The leaves were chopped into pieces, washed separately with water three times and air-dried at room temperature for duration of two weeks. The dried samples were pulverized using a grinding mill (Christy Lab Mill, England).

3.3 HYDROETHANOLIC (50%) CRUDE EXTRACT PREPARATION

One hundred gram of powdered sample of *A. conyzoides* leaves were macerated using 1000 mL of 50% ethanol. It was shaken continuously with a shaker (Gallenkamp, England) for 24 hours at 25 ^{o}C. The supernatant was filtered after centrifuging with Homef LC-30 centrifuge for 20 minutes at a speed of 1106 x g in 50 mL centrifuge tubes at room temperature. The residue was resuspended in 1000 mL of 50% ethanol

on a shaker for another 24 hours. Both supernatants were pooled together and concentrated at 50 ºC using a rotary evaporator (Buchi Rotavapor R- 205, Switzerland). A vacuum freeze dryer (YK-118, Taiwan) was used to lyophilized frozen samples and designated as *A. conyzoides* crude extract.

3.3.1 Fractionation of A. conyzoides leaf Extract

Fractionation of the hydroethanolic crude extracts of *A. conyzoides* leaves was carried by solvent – solvent extraction using ethyl acetate followed by methanol in a separating funnel. A mass of 15 g of the crude extract was dissolved in 150 mL of ethanol: water (50:50) mixture and successively partitioned with ethyl acetate followed by methanol fraction. For each fractionation step, extraction was performed twice with 300 mL of the solvents. The remaining portion was designated as aqueous extract. The three fractions namely: Ethylacetate, methanol and aqueous fractions were then concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor R-205, Switzerland) at temperatures of 69ºC, 77ºC and 79ºC respectively. Ethylacetate and methanol were dried at a temperature of 25 ºC while the remaining aqueous extract was lyophilized using a vacuum freeze dryer (Labconco, England). The fractions were stored at -20 ºC until required for use.

3.4 IN VITRO ASSESSMENT OF CRUDE EXTRACTS AND FRACTIONS OF *A. CONYZOIDES*

3.4.1 DPPH radical scavenging assay

DPPH is reduced to yellow colour when there is a reaction between an antioxidant compound (capable of donating hydrogen) and DPPH (a stable N-centred radical

purple in colour). This colour change is measured at an absorbance of 517 nm using a UV/Vis light spectrophotometer (Govindarajan *et al.,* 2003).

3.4.1.1 Procedure

Crude extract of *A. conyzoides* and fractions were prepared by dissolving 10 mg of each of the freeze-dried samples in 1 mL of 50% hydroethanolic solvent, ethylacetate and methanol solvents respectively. Also, stock solutions of 10 mM of standard (Ascorbic acid) and 0.5 mM of DPPH were prepared by dissolving 2.2 mg of ascorbic acid in 1 mL and 3 mg of DPPH in 15 mL absolute methanol. Prepared solutions were then vortexed until all molecules were completely dissolved. Solution of DPPH was kept in a dark room immediately to prevent photo-bleaching in light.

All the different extracts were serially diluted in 50% ethanol to obtain a concentration range of 0.156–10 mg/mL in a 96 well plate. The reaction mixture was made up of 100 μL of 0.5 mM 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), and 100 μL of each concentration of the test sample. For positive control or standard, ascorbic acid was used at a concentration range of 0.156–10 mM in methanol. Some of the solvents were used as blanks. Triplicate experiments were performed. The plates were covered with aluminium foil, shaken gently and kept in the dark for 20 minutes after which the absorbance was read on a Tecan-PC infinite M200 Pro Plate reader (Austria), at the absorbance wavelength of 517 nm. Percentage scavenging activity was determined by;

$$\% \text{ Scavenging} = \frac{[\text{Absorbance of blank (OD0)} - \text{Absorbance of test (OD1)}] \times 100}{\text{Absorbance of blank (OD0)}}$$

The mean percentage antioxidant activity for the triplicate experiment was plotted for the standard and samples and their effective concentration at 50% (EC_{50}) values calculated (Govindarajan *et al.,* 2003).

3.4.2 TOTAL PHENOL CONTENT DETERMINATION

All phenolic compounds contained in a mixture or extract are oxidized by Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent contains phosphotungstic acid and phosphomolybdic acid and changes colour to a mixture of blue oxides of tungstate molybdate, after phenols have been oxidized. The maximum absorbance for the blue colouration produced is 750 nm, and is proportional to the total quantity of phenolic compounds originally present (Muchuweti *et al*., 2007).

3.4.3.1 Procedure

Stock solutions of the crude extracts and fractions was prepared by dissolving 10 mg of each of the freeze-dried samples in 1 ml of 50% hydroethanol, ethylacetate and methanol solvents. A stock solution of 5 mg/mL of standard (gallic acid) was prepared. The method reported by Ghasemi *et al.* (2009) with slight modification was adopted for the determination of the total phenolic content of the extracts. A six-fold serial dilution was carried out on the gallic acid standard to obtain six different concentrations 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mM. An ethanol blank, that is, absolute ethanol without gallic acid, was also prepared. A three-fold serial dilution was also carried out on each extract to obtain three different concentrations (10, 5, 2.5 mg/mL). A volume of 10 µL of each sample and gallic acid dilutions were aliquoted into a 2.0mL eppendorf tube. 790 µL volume of distilled water were then added, followed by the addition of 50 µL of Folin-Ciocalteau reagent. The mixture was mixed

thoroughly by vortexing for 5 seconds. This was followed by incubation of the tubes in darkness at 25 ^{o}C for eight minutes.

Afterwards, 150 µL of 7% sodium carbonate solution was added to each tube, mixed thoroughly by vortexing for five seconds and further incubation of the tubes in darkness at room temperature was done for two hours. After the two-hour incubation, a volume of 200 µL of each reaction system of extract and gallic acid standard dilutions were aliquoted into wells on a 96-well plate in triplicate and read at an absorbance of 750 nm using microplate spectrophotometer. A graph of absorbance against concentration was plotted for the gallic acid standard. The concentration of phenolics in each sample was determined using the gallic acid standard plot and the gallic acid equivalence for each extract also calculated.

3.4.3 FT-IR ANALYSIS

Fourier transform infrared spectrophotometer (FTIR) was used to identify the various functional groups available in the compounds contained in the extract. Dried powders of the extracts and fractions were used in the analysis. 10 mg each of dried extract powder were encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. Using a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}, the powdered sample of each extract and fraction were loaded in FTIR spectroscope and readings taken.

3.4.4 GC-MS ANALYSIS

GC-MS analysis was performed using Perkin Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column coated with poly- methyl silicon

(0.25nm x 50m) and the conditions were as follows: Temperature programming from 80-200°C held at 80°C for 1 minute, rate 5°C/min and at 200°C for 20min. Field ionization detector (FID) Temperature of 300°C, injection temperature of 220°C, carrier gas nitrogen at a flow rate of 1 ml/min, split ratio of 1:75. Gas chromatography mass spectrum was conducted using GCMS-QP 2010 plus (Shimadzu Japan) with injector temperature of 220°C and carrier gas presence of 116.9 KPa. The column length was 30 m with a diameter of 0.25mm and flow rate of 50ml/min. Elutes were automatically passed into a mass spectrometer with a detector voltage set at 1.5 Kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra bank. Component Identification: Chemical constituent of the extract was identified by matching the peak with computer NIST libraries and confirmed by those comparing mass spectra of the peaks and those from literature.

3.4.5 QUALITATIVE PHYTOCHEMICAL SCREENING

Methods described by Ayoola *et al*. (2008) was used in determining the phytochemicals present in the crude extract of *A. conyzoides* and it fractions with a slight modification. Methods for the respective phytochemicals are as follows:

3.4.5.1 Terpenoids

The Salkowski's test indicates the presence of triterpenoid nucleus. In this test, about 200 mg of the powdered plant material was cold extracted by shaking with 10 mL chloroform and filtered. H_2SO_4 was carefully added drop-wise down the side of the test tube. The formation of cherry-red or brown ring at the chloroform-sulphuric acid interface indicated a triterpenoid nucleus (Evans, 2009).

3.4.5.2 Saponins

A volume of 1mL of water was added to 10 mg of extracts. It was then shaken continuously for 60 seconds. The presence of saponins was demonstrated by a stable persistent froth.

3.4.5.3 Tannins

About 200 mg of powdered plant material was boiled with 20 mL of water for 4 minutes. The mixture was cooled at room temperature and then filtered. The filtrate was adjusted to of 20 mL volume with distilled water. To 1 mL aliquot of the aqueous crude extract, 10 mL of water was added and 3 drops of 1 % lead acetate reagent also added. The colour and amount of precipitates formed, were noted and recorded. The procedure was repeated using 4 drops of 1 % ferric chloride in one instance and also 5 drops of 1 % gelatin solution in another instance (Evans, 2009).

3.4.5.4 Alkaloid

About 500 mg of the powdered plant material was cold-extracted with 20 mL ammoniacal alcohol (ammonia/alcohol 1:9), filtered and evaporated to dryness in a petri dish over a water bath. The residue was extracted with 1% H_2SO_4, filtered with the aid of a filter paper. The filtrate was made distinctly alkaline with dilute NH_3 solution. This was then shaken with chloroform in a separating funnel and the two immiscible layers separated. The chloroformic layer was evaporated and the residue dissolved in 1% H_2SO_4. 5 drops of Dragendorff‟s reagent was added to the extract above and the formation of orange-red precipitates, if any, was noted and recorded (Evans, 2009)

3.4.5.5 Flavonoids

Aqueous extracts were prepared by boiling about 200 mg of the powdered plant material with 20 mL distilled water. This was cooled and filtered. Rectangular strips of Whatman No. 4 filter papers were immersed in the extracts and dried at room temperature. The paper strips were exposed to fumes of strong NH_3 in fume hood and the formation of an intense yellow colour was recorded. A confirmation test was carried out by exposing the yellow coloured filter paper strips to concentrated HCl fumes. The disappearance of the intense colouration validates the presence of flavonoids in the samples (Evans, 2009).

3.4.5.6 Glycoside

About 300 mg each of the powdered plant sample, was heated with 10mL dilute H_2SO_4 on water bath for 4 minutes and allowed to cool. The mixture was filtered and the filtrate made distinctly alkaline with about 3 to 4 drops of 20 % NaOH. Two millilitres each of Fehling's solution A and B were then added gently to the filtrate, and the mixture was heated for 4 minutes on the water bath. The presence of brick red precipitate suggested that glycosides are present (Evans, 2009).

3.4.5.7 Phytosterol

The Lieberman-Burchard's test which is a modification of the Salkowski's test is performed to test for the presence of phytosterols. About 200 mg of the powdered plant material was cold extracted by shaking with 10 mL chloroform and filtered. 2 mL solution of acetic anhydride was added to the extract in a test tube and mixed thoroughly and drops of concentrated H_2SO_4 were cautiously poured down the side of the test tube. The formation of violet to blue colouration at the upper acetic anhydride layer indicated steroidal nucleus (Evans, 2009).

3.5 IN VIVO ASSESSMENT OF HEPATOPROTECTIVE EFFECT OF CRUDE EXTRACTS AND FRACTIONS OF *A. CONYZOIDES*

Sprague – Dawley (SD) male rats of weighing between 170 and 200 g were used in the experiment. Animals were obtained from the School of Medical Sciences, University of Ghana, Korle Bu, and housed at the Animal Holding Facility of the Department of Biochemistry and Biotechnology, KNUST. They were housed in cages made with aluminium and well embedded with wood shaving. The animals were kept in a standard condition and given access to feed, as well as, water. Animals were grouped based on their body weight and identified with a unique mark on their tail.

3.5.1 Evaluation of Hepatoprotective Activity

A total of 30 animals were used in this study with six groups (five animals in each group). Animals in Group I served as the control, thus were given freshly distilled water only throughout the study. CCl_4 was administered at 1 ml/kg animals in Group II to Group VI on the 2nd and 3rd day of experiment. Group II animals were maintained as CCl_4 control without any treatment, while group III animals were pre-treated with 100 mg/kg of silymarin. Group IV, V and VI animals were pre-treated with 250 mg/kg body weight of crude extracts, methanolic and aqueous extract respectively. Treatment for all groups was perform through oral administration from day one to day seven with a concurrent administration of CCl_4 (1 ml/kg b.wt) on the 2^{nd} and 3^{rd} day (Arthur *et al*., 2012). The doses for the extract was selected based on subchronic toxicity assessment of crude extract of *A. conyzoides* that established 250 mg/kg as the safest dose (Acheampong *et al.,* 2015)

3.5.2 Serum Preparation, Enzyme Assay and Liver Isolation

All animals were sacrificed on the 8th day following an overnight fasting. Animals were exposed to ether. Incisions were quickly made in the neck region of the animals and blood samples collected from the heart into gel activated tubes for biochemical analyses such as ALT, ALP, bilirubin (total and direct), total cholesterol,urea, creatinine and triglycerides. All biochemical analyses were performed using Fortress Diagnostics Reagents (UK) following manufacturer's instructions. All measurements were performed using Respons 920 fully Automated Biochemistry Analyzer.

3.5.3 Effect of Treatment on Liver and kidney Weight

The liver and kidney of rats in the respective groups sacrificed were excised, washed in a buffed saline and weighed. Absolute liver weights of organs were obtained The formula below was used to calculate the relative weights:

$$\text{Relative organ weight} = \frac{\text{Absolute Organ Weight X 100 \%}}{\text{Body weight as sacrifice}}$$

3.5.4 Liver specimen preparation

The organs were dissected into 2 parts, one part placed in formalin for histopathological examination and other placed in buffered saline for antioxidant enzyme determination.

3.5.5 Histopathology Analysis

A piece of liver and kidney tissue were preserved in a 10% formalin solution. Afterward, the liver and kidney samples were then washed with tap water and diluted with methyl, ethyl and absolute ethyl to enhance dehydration. Paraffin beeswax tissue

blocks prepared were used for portioning the samples into a thickness of 4 µm. Acquired portions of tissue were placed on a glass slides, deparaffinised and stained using haematoxylin and eosin.

Afterward, samples were examined with a light microscope (Carl Zeiss mark Axio imager A2 model). Scoring of specimens were done after evaluating five microscopic areas of the tissue. Liver and kidney injuries were classified based on the following criteria: vascular congestion, lobular inflammation, glomerusclerosis, tubular hyalinization, cellular degeneration and tubular necrosis and fibrosis. Examination of specimen were done by a trained pathologist, Dr. Babatunde Duduyemi, Department of Anatomy, SMS, KNUST, Kumasi.

3.5.6 Determination of Oxidative stress markers in liver and kidney tissues

TNG buffer (4.5 ml) at pH of 7.4 was added to each organ sample and homogenized. The homogenized sample was then centrifuged at 4000 x g for 20 minutes in a refrigerated centrifuge, afterward supernatant was taken and preserved for antioxidant enzyme analysis. Microtiter plate reading was performed thrice for each sample.

3.5.6.1 Total Protein determination

Method described by Lowry *et al.* (1951) was slightly modified for the determination of total protein content in the samples. Bovine serum albumin standard curve was prepared for a series of dilutions of 0, 1, 2.5, 10 and 20 µg/well with a final volume of 100 µl. Samples were also diluted within the standard range (0-25 µg/100 µl) and 100 µl added to each well. After diluting and transferring standards and samples to the

microplate, a volume of 200 µl of Biuret reagent was added to each well and thoroughly mixed. 20 µl of 1.0 N Folin Ciocalteu's reagent was added to each well after incubating the sample mixtures at room temperature for 15 minutes. Absorbance measurement was read at 650 nm using ELx808 Microplate Reader (BioTek Instruments, Winooski, VT)

3.5.6.2 Lipid peroxidation

The presence of polyunsaturated fatty acid (PUFA) degradation was measured by the quantity of MDA formed. MDA is mostly involved in thiobarbituric acid (TBA) assay to indicate the presence of PUFA oxidative damage. Method described by Health and Packer (1968) was used to determine the levels of MDA formed. 3 ml of the mixture (3 ml 20 % TCA containing 0.5 % TBA) was added to 1 ml of homogenate sample and heated for 30 minutes at 95 ^{o} C. It was then cooled immediately after heating and centrifuge for 10 minutes as a speed of 5000 x g. Samples were read at 532 nm absorbance and later read again at 600 nm. The amount of MDA was calculated from the formula below:

$$nmol\ MDA/mg\ protein = \frac{Absorbance_{532\ nm} - Absorbance_{600\ nm}}{155 \times total\ protein} \times 10^{6}$$

3.5.6.3 Catalase (CAT) Assay

CAT has the potential to hydrolyse H_2O_2 and hence prevent the formation of chromate acetate by H_2O_2 (Sinha, 1972). A volume of 0.4 ml of H_2O_2 and 1 ml of phosphate buffer was added to 0.1 ml of homogenate sample and incubated at room temperature for 5 minutes. To terminate the reactions, a volume of 2 ml dichromate acetic mixture

was added to the sample and subsequently read at absorbance of 620 nm. CAT activity was determined by the equation below:

$$mUnit\ of\ CAT\ activity/mg\ protein = \left(\frac{Absorbance_{620\ nm}}{39.4\ \times weight\ of\ protein}\right) \times 1000$$

3.5.6.4 Superoxide dismutase (SOD) activity measurement

SOD prevents the autoxidation of adrenaline to adrenochrome (Misra and Fridovich, 1972). A volume of 150 µl of ice-cold chloroform was added to 500 µl of the homogenate sample. 750 µl ethanol was then added and centrifuge for 20 minutes.

500 µl of supernatant was added to 1 ml carbonate buffer, afterward 0.5 ml EDTA and 0.05 ml of adrenaline solution were added. The percentage of adrenaline autoxidation inhibited was calculated as:

$$\%\ inhibition = \left(\frac{Absorbance\ _{test}\ - Absorbance\ _{blank}}{Absorbance_{test}}\right) \times 100$$

Activity of SOD was also determined using the equation below:

$$Units\ of\ \ SOD\ activity/mg\ protein = \left(\frac{\%\ inhibition}{50\ \times weight\ of\ protein}\right)$$

3.5.6.5 Reduced Glutathione (GSH) Assay

Method described by Ellman (1959) was used to determine the level of GSH. A volume of 100 µl of homogenate was added to 2.4 ml of EDTA and allowed to cool for 10 minutes at 4 ^{o}C. 1 ml of supernatant was added to a mixture containing 50 µl of 10 mM DTNB solution and 2 ml of 0.4M Tris buffer and incubated at room temperature for 5 minutes. Absorbance was spectrophotometrically read at 412 nm.

GSH concentration was determined using the curve, where *x* is the absorbance at 412 nm and y the concentration of GSH at µmol/mg protein.

$$y = 0.0004\,x + 0.0026$$

3.5.6.6 Myeloperoxidase (MPO) Assay

O-dianisidine method by Şenoğlu *et al*. (2009) was used to determine the enzyme concentration. 5 ml of 0.02 M o-dianisidine and 3 ml phosphate buffer was added to 50 µl of homogenate. Absorbance was read at 460 nm. MPO specific activity was measured.

$$Unit\ of\ MPO/mg\ protein = \left(\frac{Absorbance_{460\ nm}/min}{0.001\ \times (total\ protein/10\ \mu l\ aliquot)}\right)$$

3.6 STATISTICAL ANALYSIS

GraphPad Prism 6 for Windows was used to analyse the data. Results were expressed as the mean ± standard error mean (SEM). Data were assessed by one- way analysis of variance followed by Newman–Keuls multiple comparison test. Values for which $P < 0.05$ were considered as statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 IN VITRO ASSESSMENT OF THE BIOLOGICAL ACTIVITIES OF CRUDE EXTRACTS AND FRACTIONS OF *AGERATUM CONYZOIDES*

4.1.1 In vitro DPPH scavenging activity

Ability of the test extracts to donate hydrogen atoms or electrons was measured using spectrophotometer. DPPH was reduced to diphenylpicrylhydrazine in all extracts and diminished the absorbance at 517 nm. EC_{50} used in the studies describes the concentration at which 50% of DPPH free radicals are effectively inhibited.

From the experiment, comparing results with the standard (ascorbic acid), it was observed that the crude hydroethanolic extract scavenged DPPH radicals more strongly with EC_{50} value of 0.14 mg/ml, followed by the aqueous fraction (0.20 mg/ml) and methanol fraction. Ethyl acetate fractions had the weakest DPPH radical scavenging potential with EC_{50} values > 5mg/ml. Generally, the difference between the various EC_{50} values for the different extracts were statistically insignificant ($p > 0.05$ for both the crude extracts and aqueous fraction) and statistically significant for the methanol fraction (Table 4.1).

Table 4.1 Comparison of EC_{50} values of DPPH scavenging of standard and extracts

	EC_{50} values (mg/ml)	
Sample/standard	**n = 3**	**P values**
Crude	0.1352 ± 0.01009	0.89
Hydroethanolic	0.1983 ± 0.04149	0.98
Methanol	3.5569 ± 2.67441	0.00
Ethyl acetate	> 5	
Ascorbic acid	0.1762 ± 0.05760	

4.1.2 Total phenol content

Folin-Ciocalteau's reagent was used to measure the total phenols content in the extracts at an absorbance of 750 nm. Comparing the crude extract, hydroethanolic, ethyl acetate and methanol fraction, the total phenol contents was high in the crude extract (0.392 mg/g) and low in the ethyl acetate fraction (0.292 mg/mL). In terms of gallic acid equivalent, the total phenol content in both the crude and ethyl acetate fractions were 7845.592 ± 2703.75 and 5842.462 ± 1123.859 respectively. GAEs (mg/g) of plant extract in crude extracts and fractions of *A. conyzoides* is shown in Table 4.2 below.

Table 4.2 Total phenolic contents standard and extracts

Sample/standard	Leaves n = 3	GAE (mg/g)	P value
Crude	0.392 ± 0.1352	7845.59 ± 2703.74	0.00
Hydroethanolic	0.380 ± 0.0470	7605.63 ± 940.53	
Ethyl acetate	0.292 ± 0.0562	5842.46 ± 1123.86	
Methanol	0.300 ± 0.0426	6009.39 ± 852.00	
Gallic acid	4.938 ± 0.0199		

4.1.3 Qualitative Phytochemical Constituent of A. conyzoides crude extract and fractions

The presence of various phytochemicals were determined in the 50% hydroethanolic crude extracts of *A. conyzoides* and its fractions. Most phytochemicals were found to be abundant in the crude extract with the ethyl acetate fraction having the least phytochemicals. The table below indicate the presence of phytochemicals in the extracts (Table 4.3).

Table 4.3 Preliminary phytochemical constituent of *A. conyzoides* crude extract and fractions

Phytochemicals	Crude extract	Ethyl acetate	Methanolic	Hydroethanolic
Alkaloid	+	-	+	+
Flavonoid	+	-	+	+
Terpenoid	+	-	+	-
Tannins	+	+	-	-
Saponin	+	+	+	+
Phytosterol	+	+	-	-
Glycoside	+	+	+	+

(+) indicate presence of phytochemicals, while (–) indicate the absence of phytochemicals

4.1.4 FTIR Spectroscopic Analysis

The chemical bonds of compounds present in the crude extract, aqueous, ethyl acetate and methanolic fractions of *A. conyzoides* were determined using Fourier Transform Infrared Spectrophotometer (FTIR). When the samples were passed into the FTIR, the functional groups of compounds present were separated based on their peak ratio. The results below confirm the functional groups present in each of the samples. The identified functional groups include Alcohol, phenol, Aldehydes, Aromatics, Alkyl halide, Alkanes, Carbonyls, Carboxylic acids, Alkenes and Alkynes. (Table 4.4-7).

Table 4.4 FTIR Peak Values of 50% hydroethanolic crude extract of *A. conyzoides* leaves.

Peak No.	Peak Value	Functional Groups
1	3265.68	Alcohol, phenol
2	2920.09	Alkanes
3	2851.13	Aldehydes
4	1560.49	Nitro compound
5	1399.22	Alkanes
6	1108.03	Aliphatic amines
7	1032.01	Aliphatic amines
8	786.14	Aromatics
9	647.11	Alkyl halide
10	616.00	Alkyl halide

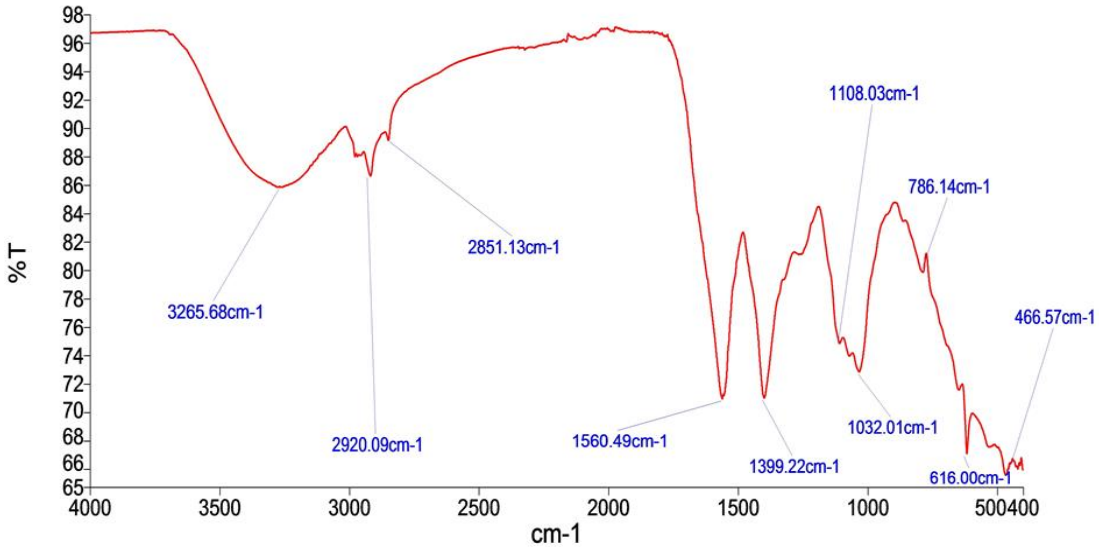

Figure 4.1 FTIR spectrum of *A. conyzoides* leaves of 50% hydroethanolic crude extract.

Table 4.5 FTIR Peak Values of aqueous fraction of *A. conyzoides* leaves

Peak No.	Peak Value	Functional Groups
1	3265.52	Alkynes
2	2980.80	Alkanes
3	2113.53	Alkynes
4	1574.69	Aromatics
5	1395.14	Alkanes
6	1106.81	Aliphatic amines
7	1030.26	Aliphatic amines
8	787.17	Aromatics
9	614.65	Alkynes
10	466.31	Alkyl halide

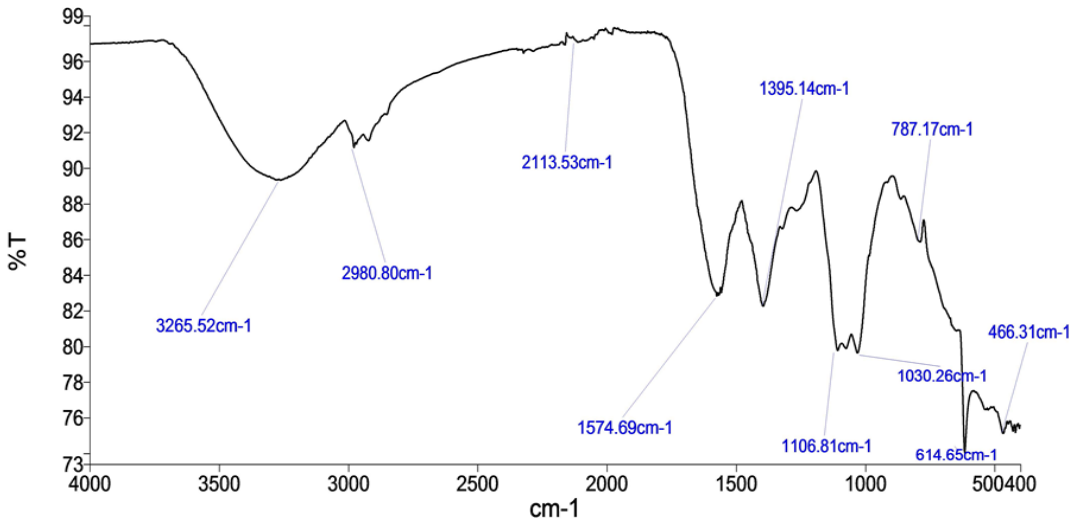

Figure 4.2 FTIR spectrum of aqueous fraction of *A. conyzoides* leaves

Table 4.6 FTIR Peak Values of ethyl acetate fraction of *A. conyzoides* leaves.

Peak No.	Peak Value	Functional Groups
1	3405.72	1˚, 2˚ Amines, Amides
2	2927.08	Alkanes
3	2855.63	Alkanes
4	1737.87	Carbonyls
5	1726.15	Carbonyls
6	1458.13	Alkanes
7	1373.28	Alkanes
8	1240.02	Alkyl halides
9	1160.99	Alkyl halides
10	1121.23	Alkyl halides
11	1044.76	Alkenes
12	940.88	Carboxylic acids

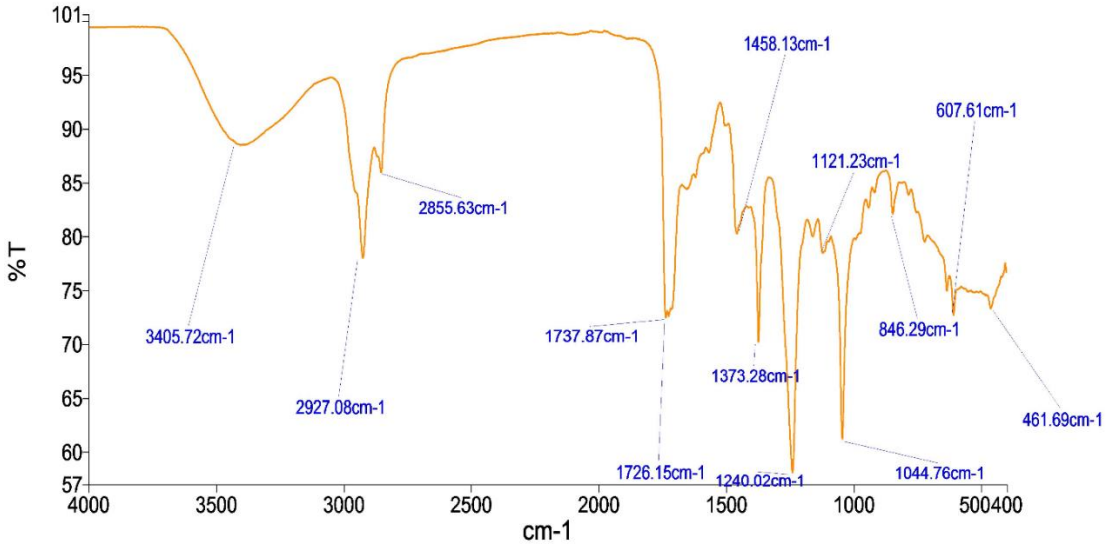

Figure 4.3 FTIR spectrum of ethyl acetate fraction of *A. conyzoides* leaves.

Table 4.7 FTIR Peak Values of methanol fraction of *A. conyzoides* leaves.

Peak No.	Peak Value	Functional Groups
1	3351.54	Alcohols, Phenols
2	2980.68	Alkanes
3	2180.16	Alkynes
4	1633.53	1˚ Amines
5	1553.98	Nitro compounds
6	1404.44	Aromatics
7	1343.72	Nitro compounds
8	1019.80	aliphatic amines
9	924.25	carboxylic acids
10	646.79	Alkynes

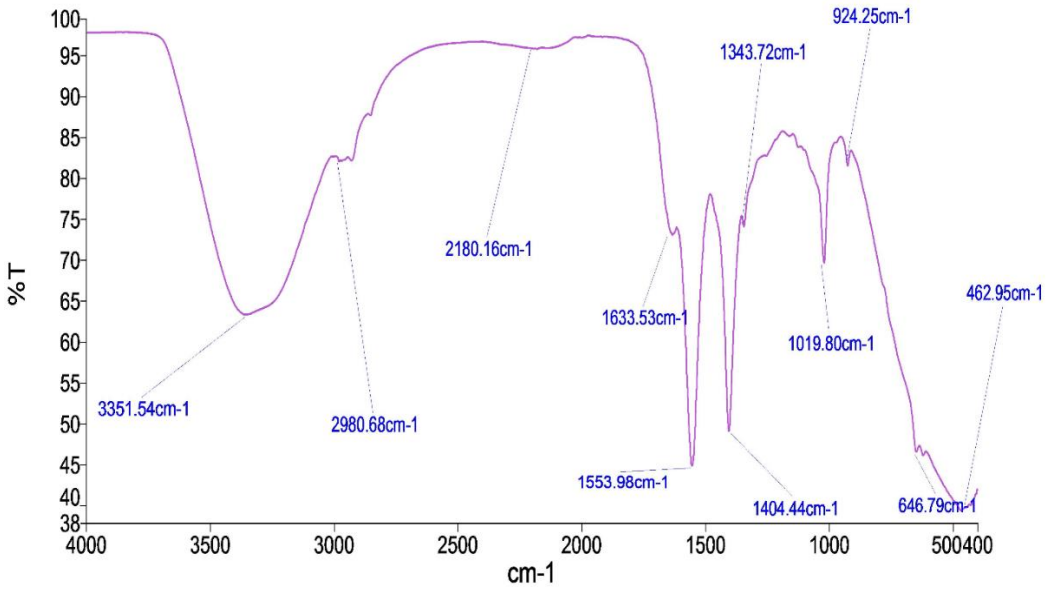

Figure 4.4 FTIR analysis of methanol fraction of *A. conyzoides* leaves.

4.1.5 Gas Chromatography Mass Spectrum Analysis of Extracts

Various compounds were found in the crude extract and its hydroethanolic and methanolic fractions. The presence of these compounds was seen to play a key role in the pharmacological functions of the plants. These compounds were determined using the GC-MS analyser which displayed all the suggested compounds present in the plant.

(Figure 4.1-4)

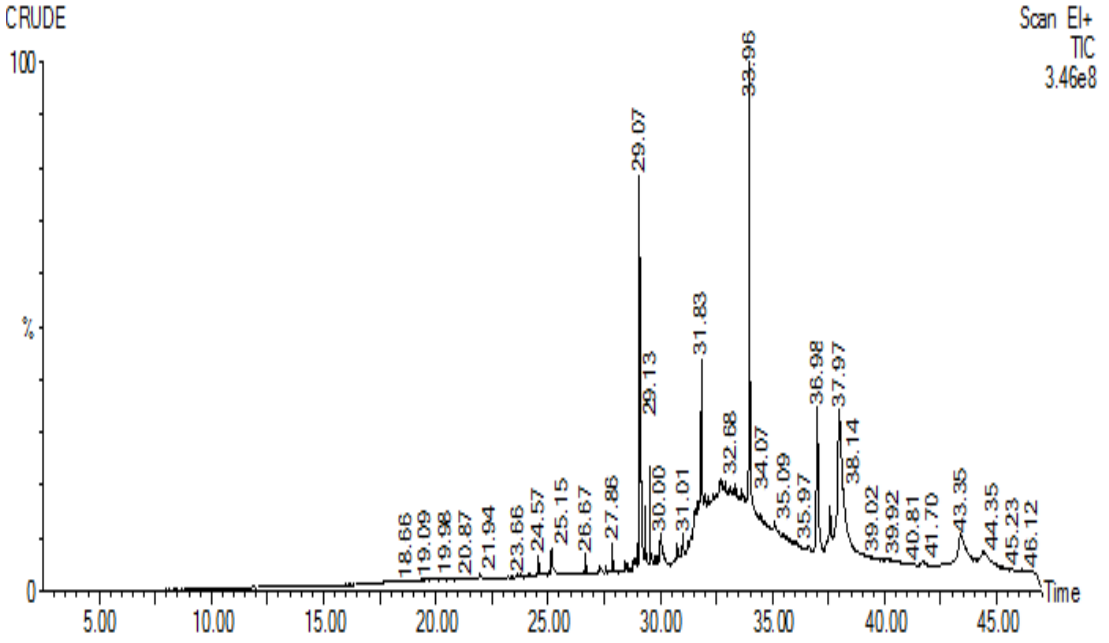

Figure 4.5 GC-CM spectrum of *A. conyzoides* crude extract of leaves showing the peaks of all compounds present.

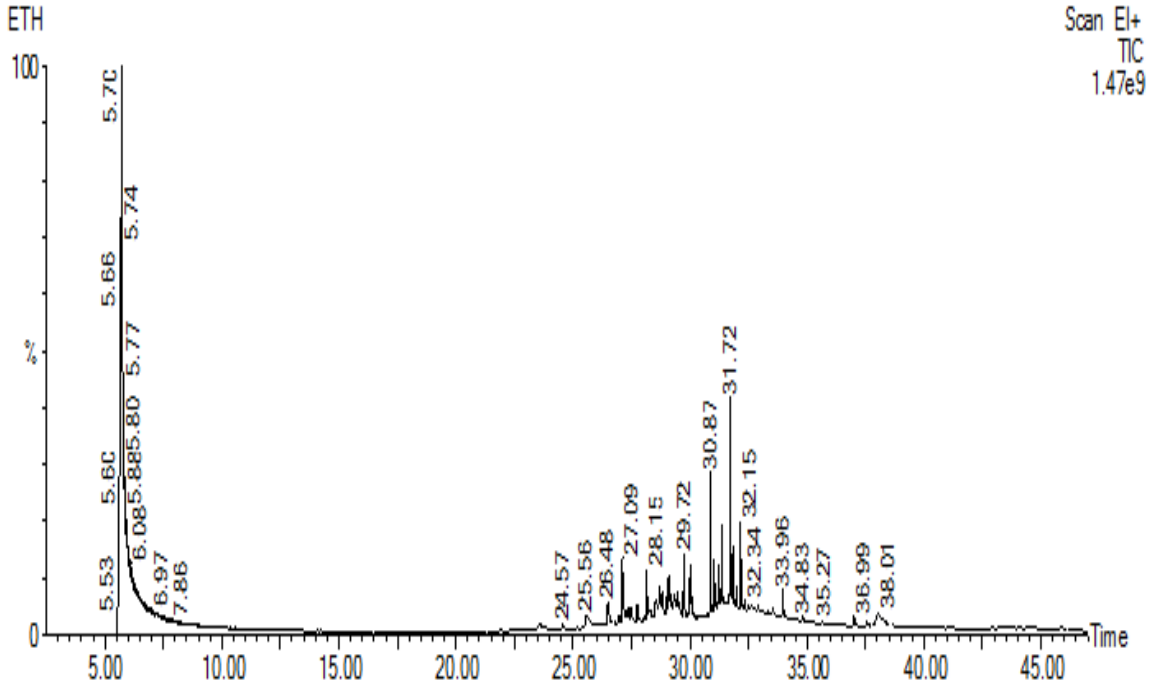

Figure 4.6 GC-CM spectrum of ethyl acetate fractions *A. conyzoides* showing the peaks of compounds present.

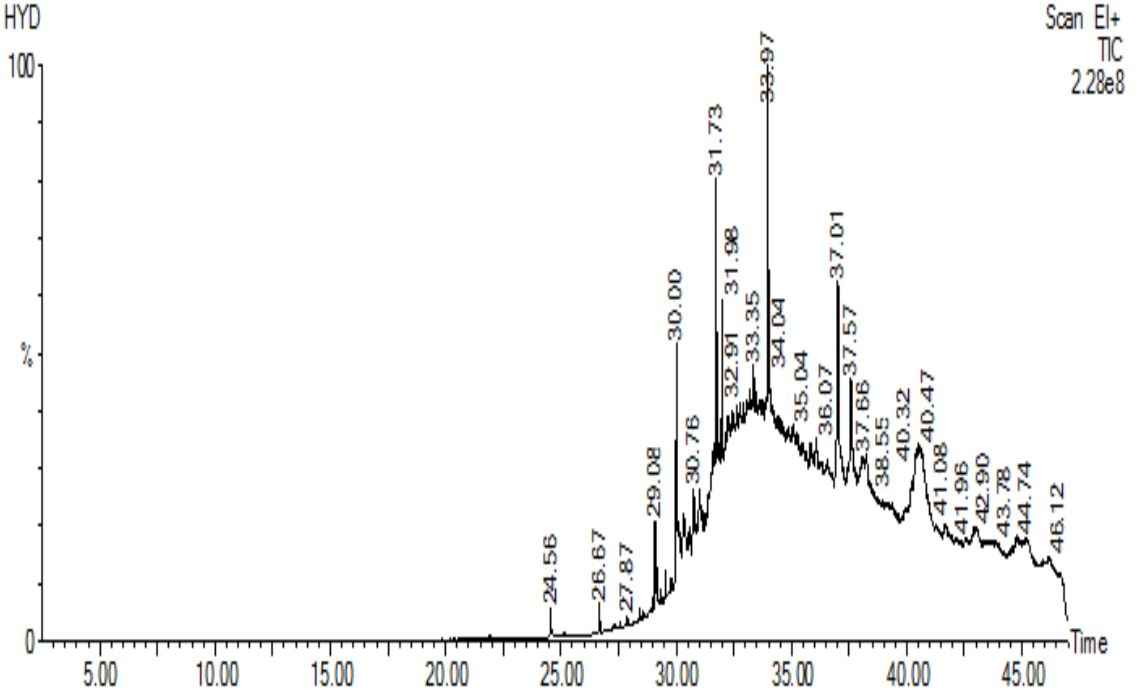

Figure 4.7 GC-MS spectrum showing peaks of compounds present in aqueous fractions *A. conyzoides*

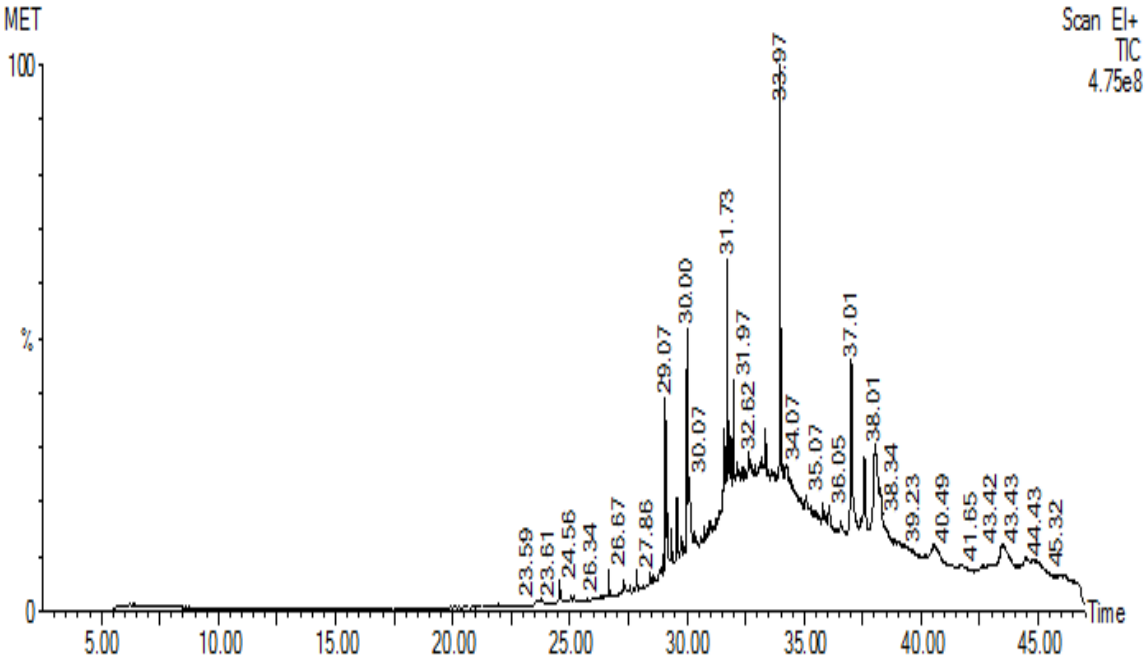

Figure 4.8 GC-MS spectrum showing peaks of compounds present in methanol fraction *A. conyzoides*

4.2 HEPATOPROTECTIVE EFFECT OF CRUDE EXTRACTS AND FRACTIONS OF *AGERATUM CONYZOIDES*

4.2.1 Effect of treatment on organ weight of rats

After blood collection, the liver and kidney were carefully resected from the abdominal region of the rat and their respective organs weight were taken. CCl_4 treatment resulted in significant increases in liver and kidney weight ($p<0.001$) compared with normal. Extract treatment restored the organ weight close to normal values (Table 4.8).

Table 4.8 Effect of treatments on body and organ weight of rats

Treatment Group	Body Weight (gm)	Kidney Weight (gm)	Relative Kidney Weight (%)	Liver Weight (gm)	Relative Liver Weight %
Normal	132.6 ± 0.50	4.04 ± 0.08	0.72 ± 0.04	0.96 ± 0.06	3.06 ± 0.09
CCl_4	176.2 ± 0.65	5.73 ± 0.20	0.80 ± 0.06	1.40 ± 0.06	3.24 ± 0.14
Silymarin	155.6 ± 0.53	4.83 ± 0.11	0.68 ± 0.05	1.06 ± 0.06	3.11 ± 0.08
CCl_4 + 250mg/kg (Crude)	162.4 ± 0.57	4.65 ± 0.14	0.67 ± 0.06	1.08 ± 0.08	2.86 ± 0.09
CCl_4 + 250mg/kg (Aqueous)	130.6 ± 0.595	4.72 ± 0.14	0.77 ± 0.06	1.00 ± 0.06	3.63 ± 0.14
CCl_4 + 250mg (Methanolic)	149.8 ± 0.34	5.17 ± 0.13	0.83 ± 0.05	1.24 ± 0.07	3.45 ± 0.11

mean ± SEM (n = 5).

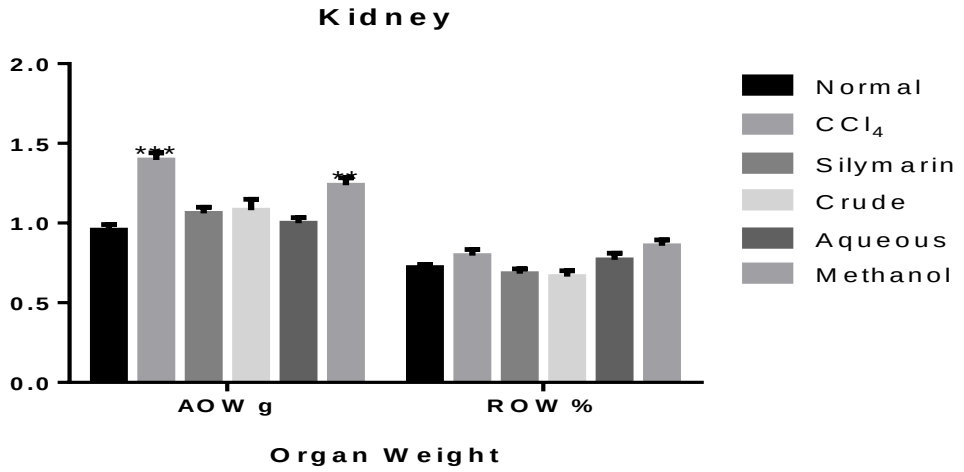

Figure 4.9 Effect of treatment on absolute and relative kidney weight of rat. Each column represents a mean±SEM of 5 animals. ** p<0.01, ***p<0.001 against Normal (ROW-Relative Organ Weight, AOW-Absolute Organ Weight)

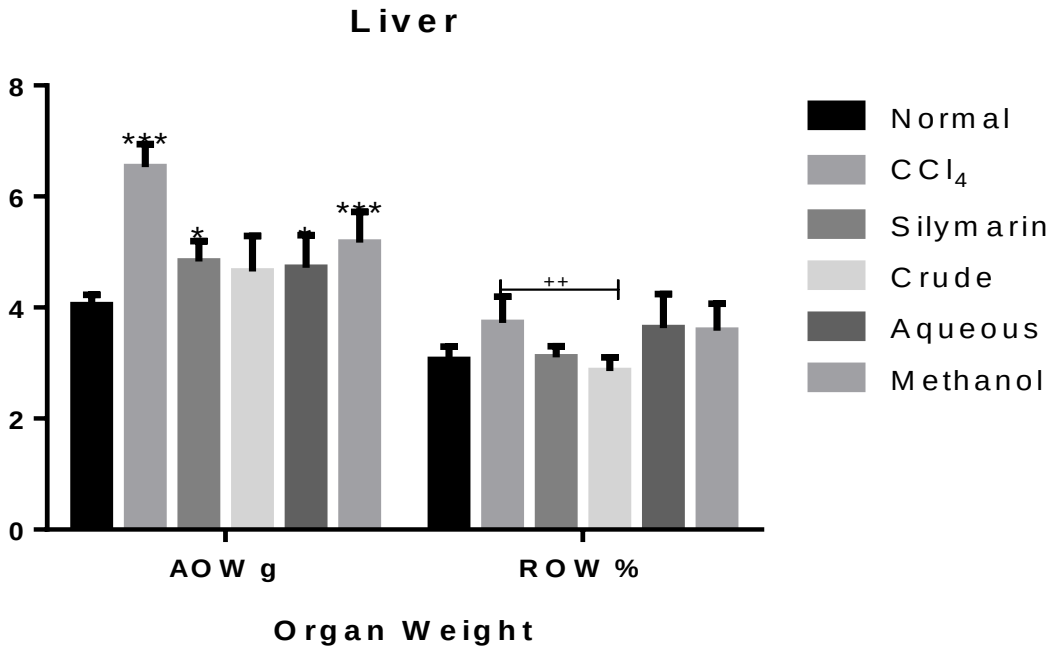

Figure 4.10 Effect of treatment on absolute and relative kidney weight in rat. Each column represents a mean±SEM of 5 animals. * p<0.05, ***p<0.001 against Normal; $^{++}$p<0.01 against CCl_4 group. (ROW-Relative Organ Weight, AOW-Absolute Organ Weight)

4.2.2 Effect of treatment on Serum Biochemistry

The biochemical profiles of the treated animals were determined as presented (Figure 4.52). The levels of liver biomarkers (AST, ALT, GGT, ALP and Bilirubin) of treated animals were determined. The extent of kidney damage of treated animals were determined by measuring the levels of Creatinine and Urea. CCl_4 treatment resulted in significant increase ($p<0.001$) in ALP, GGT and Bilirubin levels against the normal. Extract treatment resulted in decreased parameters to near normal. The increased in creatinine level due to CCl_4 treatment was restored to near normal ranges (Figure 4.11).

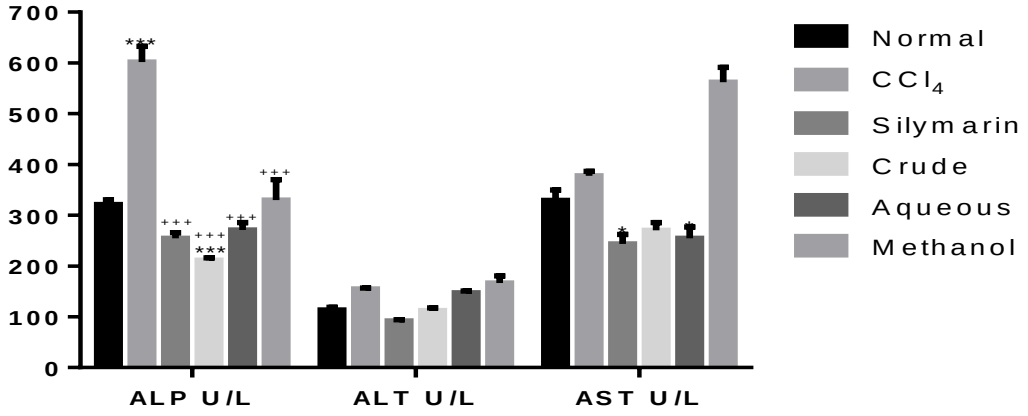

Figure 4.11 Effect of treatment on liver enzymes of rats. Each column represents a mean±SEM of 5 animals. * $p<0.05$, ***$p<0.001$ against normal; $^{+++}p<0.001$ against CCl_4 (ALP- Alkaline Phosphatase, ALT- Alanine Aminotransferase and AST- Aspartate Aminotransferase).

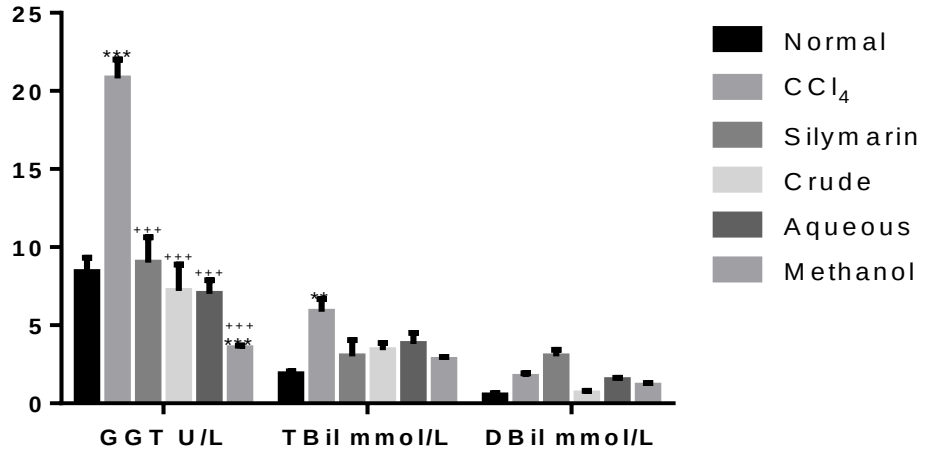

Figure 4.12 Effect of treatment on liver markers of rats. Each column represents a mean±SEM of 5 animals. **p<0.01, ***p<0.001 against normal; $^{+++}$p<0.001 against CCl_4 (GGT- Gamma-Glutamyl Transferase, TBIL-Total bilirubin, DBIL- Direct bilirubin)

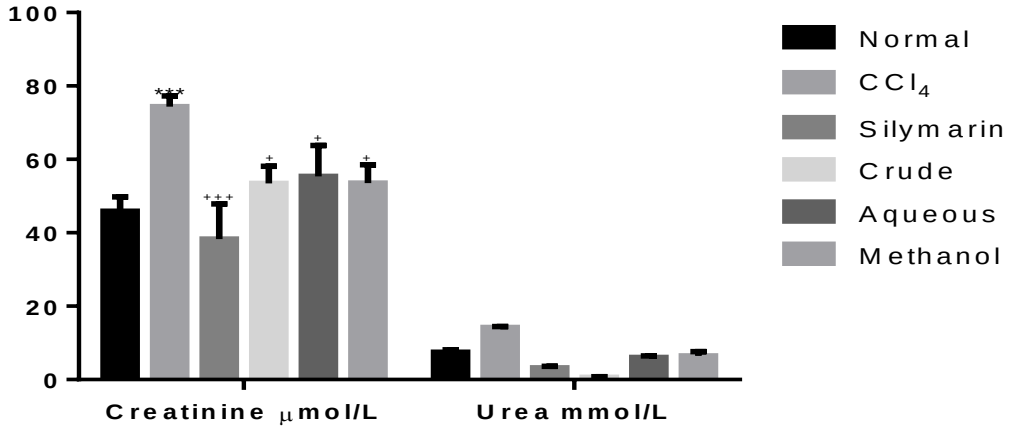

Figure 4.13 Effect of treatment on kidney profile of rats.

Each column represents a mean±SEM of 5 animals. ***p<0.001 against normal; $^{+}$p<0.05, $^{+++}$p<0.001 against CCl_4

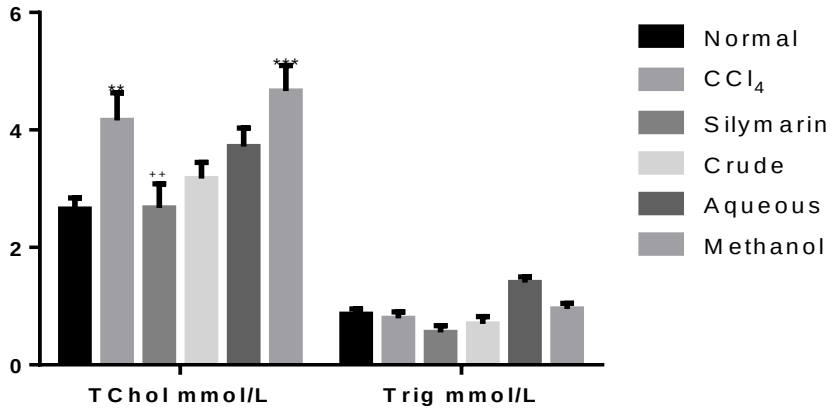

Figure 4.14 Effect of treatment on lipid profile of rats. Each column represents a mean±SEM of 5 animals. ** p<0.01, ***p<0.001 against normal; $^{++}$p<0.001 against CCl_4 (TChol- Total cholesterol, Trig – Triglyceride)

4.2.3 Percent Protection

Percent protection of silymarin, crude extract, aqueous and methanol fractions of *A. conyzoides* is shown below (Figure 4.8). These were calculated using the principal indices of liver protection (relative liver weight, ALT, ALP, GGT and Bilirubin). Crude extract offered better protection to CCl_4 induced liver damage whch was comparable to the standard drug silymarin. Methanol extract did not offer any significant protection.

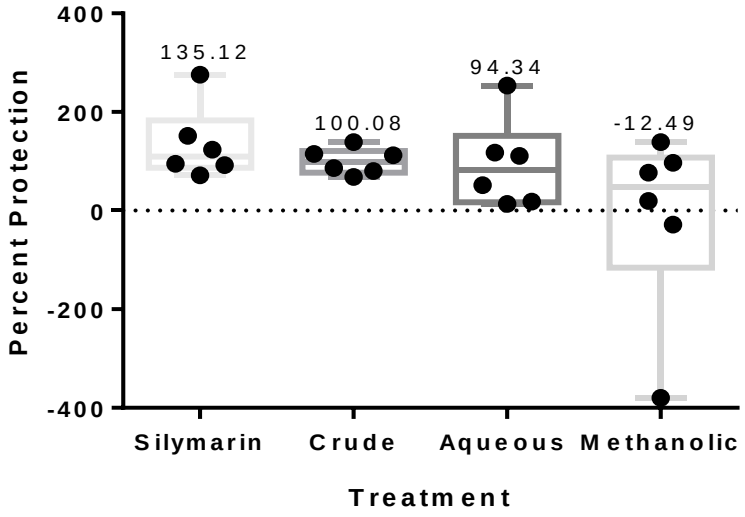

Figure 4.15 Percent Liver Protection of Silymarin and *A. conyzoides*

4.2.4 Histopathology Examination

Animals in the normal group showed regular cellular structure of the liver during histopathological examination. No morphological changes were identified in the liver. Mild lobular active inflammation was observed in the liver of animals with Silymarin treatment. CCl_4 treated group showed ballooning of hepatocytes, moderate congestion and moderate necroinflammation of the liver. Fibrosis with architectural distortion of the liver was also observed in this group. Animals treated with *A. conyzoides* crude extract showed congestion of portal tract with no significant inflammation in the liver. Group treated with aqueous fraction of *A. conyzoides also* showed lobular inflammation of the liver with focal interface, however no fibrosis was observed. Methanol fraction treated group showed mild portal congestion of the liver without evidence of necrosis (Table 4.16).

Table 4.9 Effect of treatment on liver and kidney histology of rats

Treatment groups	Liver cells	Kidney Cells
Normal	No morphological changes	No morphological changes
CCl_4	Mild lobular active inflammation	Tubular hyalinization and Glomerusclerosis
Silymarin	Mild lobular active inflammation	Congestion and mild interstitial inflammation
CCl4 + 250mg/kg (Crude)	No significant inflammation	No significant inflammation or congestion
CCl4 + 250mg/kg (Aqueous)	Stage 1 fibrosis with lobular inflammation	Mild interstitial inflammation
CCl4 + 250mg/kg (Methanolic)	Mild congestion, no inflammation	Mild interstitial nephitis

For histological observation of kidney cells, normal group of animals showed no pathological changes. In this group, normal architecture of the kidney was observed. CCl_4 treated group showed tubular hyalinization, glomerusclerosis, with interstitial inflammation of kidney. Congestion and mild interstitial inflammation of the kidney was observed in animals with Silymarin treated group. Animals in *Ageratum* crude extract treated group showed no inflammation or congestion in their kidney as shown in Table 4.9. Mild interstitial inflammation was recorded in the kidneys of rats treated with Hydoethanolic fractions of *Ageratum.* Animals treated with methanol fractions of *Ageratum* showed kidney with mild interstial nephitis (Figure 4.17).

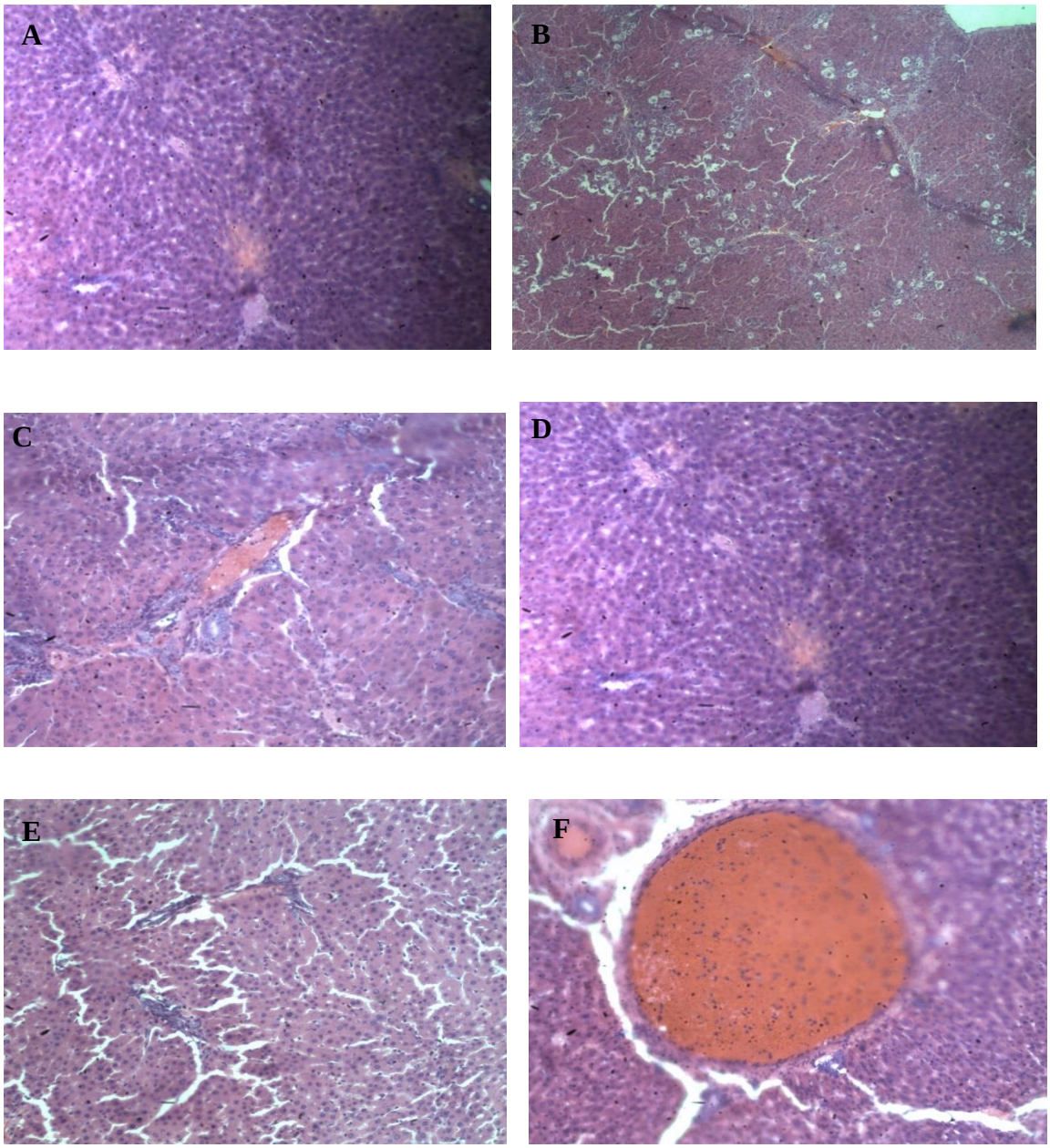

Figure 4.16 Effect of treatment on liver histology. A- Normal group showing no morphological changes; B-CCl_4 group showing ballooning of hepatocytes, moderate congestion, and moderate necroinflammation with bridging fibrosis; C-Silymarin group showing mild lobular active inflammation; D-Crude extract group showing congestion of portal tract, no significant inflammation; E-Aqueous fraction treated group showing Lobular inflammation with focal interface hepatitis / Stage 1 fibrosis; F-Methanolic fraction treated group showing mild congestion, no inflammation

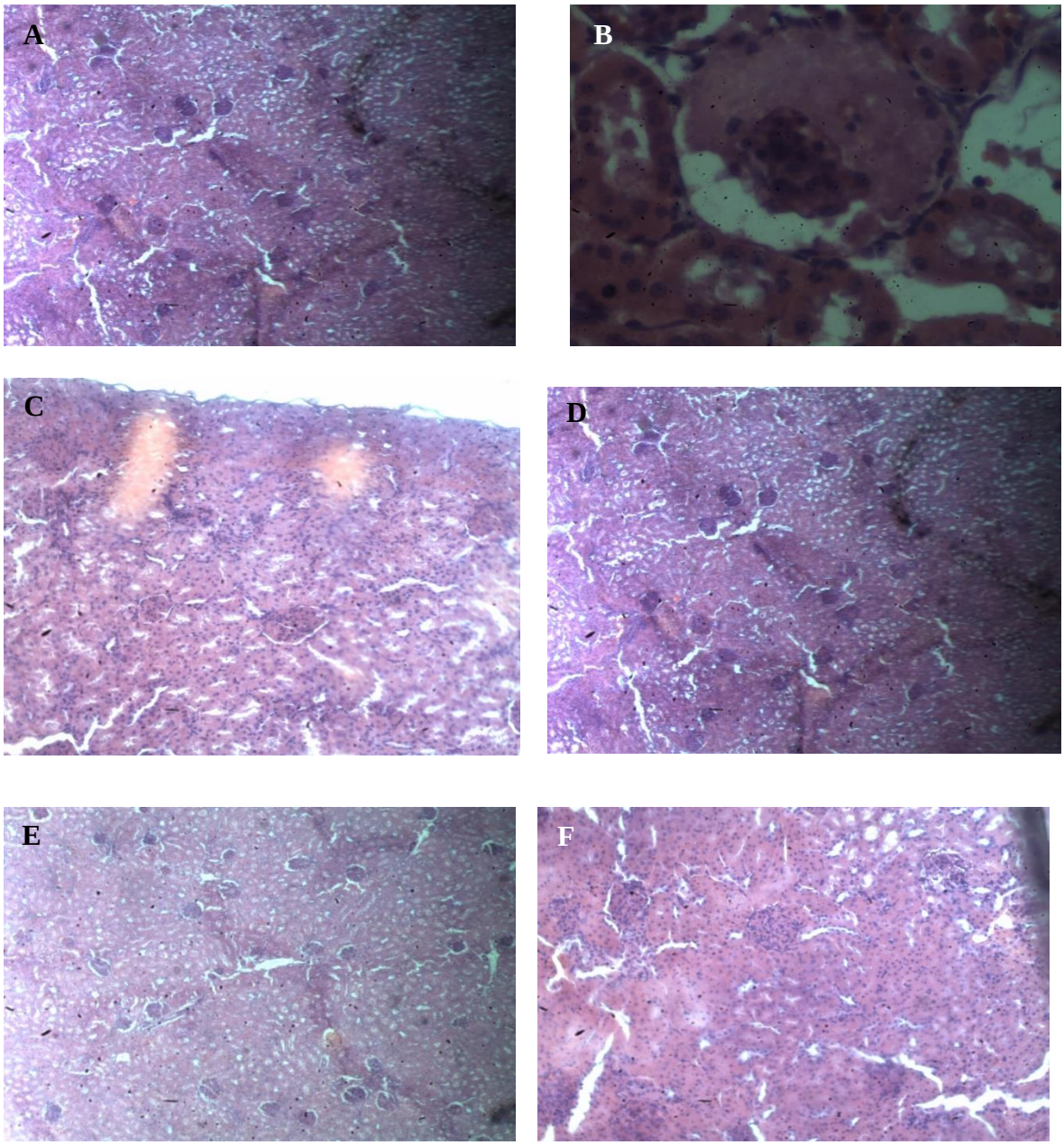

Figure 4.17 Effect of treatment on kidney histology. A-Normal group showing no morphological changes; B-CCl_4 group showing Tubular hyalinization. Glomerusclerosis. Interstitial inflammation; C-Silymarin group showing Congestion, mild interstitial inflammation; D-Crude extract group showing no inflammation or congestion; E-Aqueous fraction treated group showing mild interstitial inflammation; F-Methanolic treated group showing no congestion or inflammation.

4.2.5 Effect of treatment on Liver and Kidney Antioxidant Enzymes

Antioxidant enzymes serve as biomarkers in monitoring the levels of ROS presence in the body. Essential antioxidants such as catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) were determined in the study. Also the levels of peroxidative products, myeloperoxidases (MPO) and malondialdehyde (MDA) were also assessed. CCl_4 administration resulted in significant decreases ($p<0.001$) in CAT, SOD and GSH levels and increases in MPO and MDA levels. Silymarin and extract treatment restored these to near normal levels. The crude *A. conyzoides* was observed to be most protective (Table 4.18).

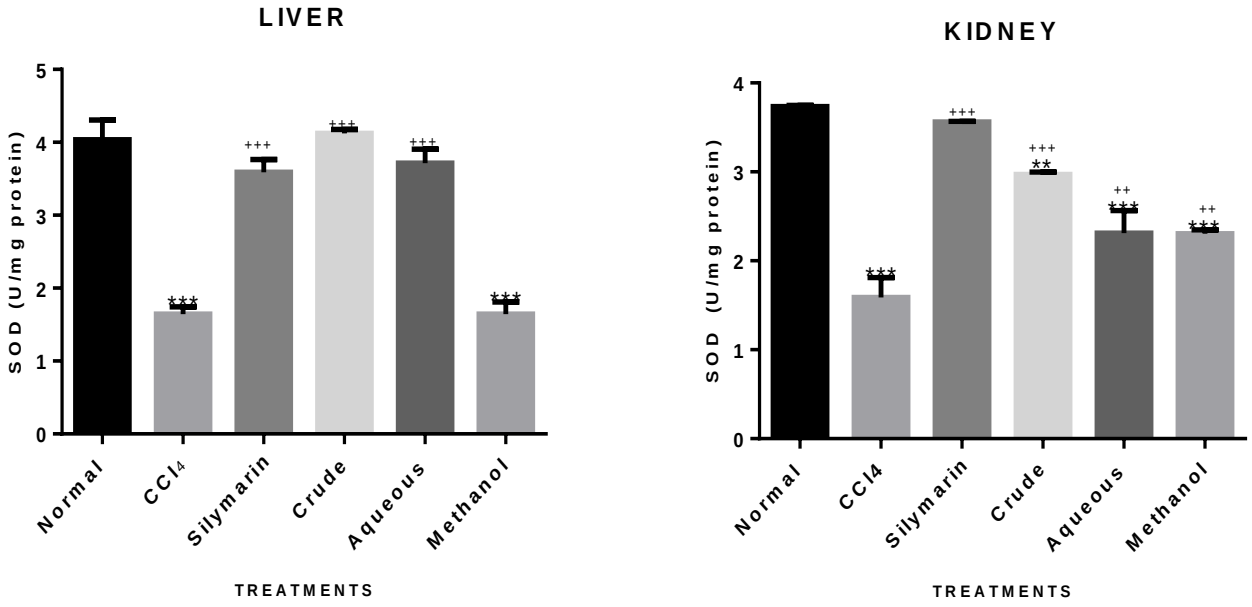

Figure 4.18 Effect of treatments on liver and kidney SOD levels. Each column represents a mean of n=5. ***$p<0.001$ against normal; $^{++}p<0.01$, $^{+++}p<0.001$ against CCl_4 group (SOD – Superoxide dismutase).

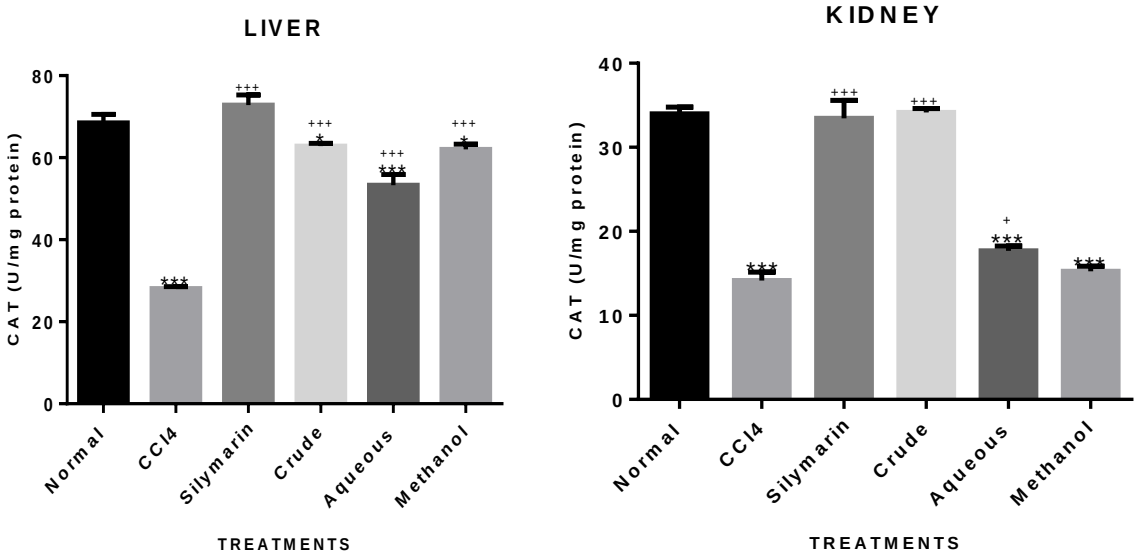

Figure 4.19 Effect of treatments on liver and kidney Catalase levels. Each column represents a mean of 5 animals. *p<0.05, ***p<0.001 against normal; $^{+}$p<0.05, $^{+++}$p<0.001 against CCl_4 group

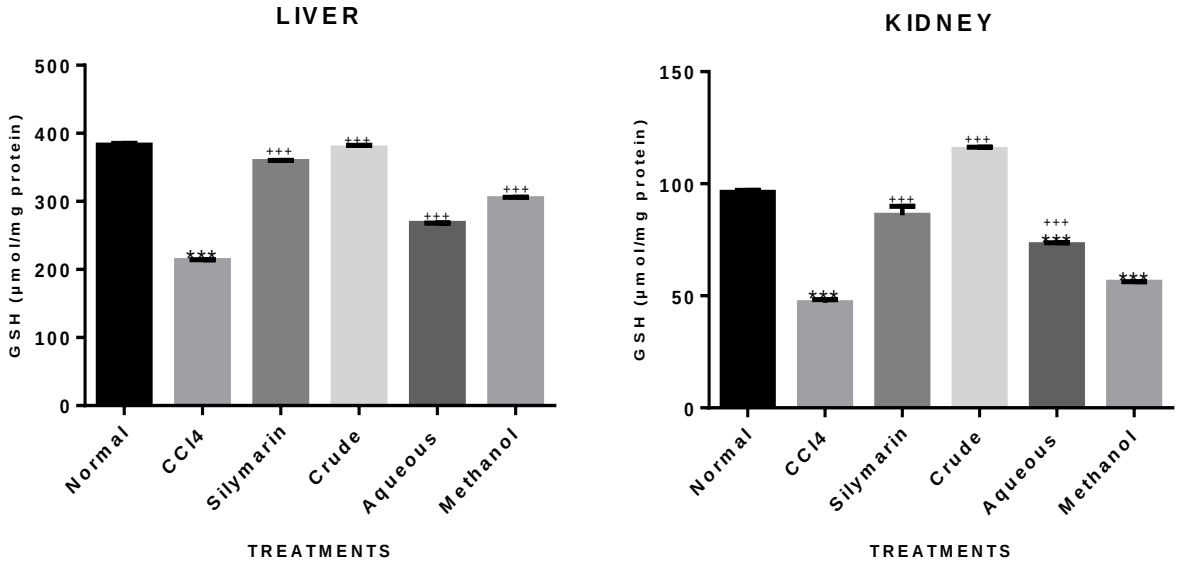

Figure 4.20 Effect of treatments on liver and kidney GSH levels. Each column represents a mean of 5 animals. ***p<0.001 against normal; $^{+++}$p<0.001 against CCl_4 group **(**GSH – Reduce Glutathione).

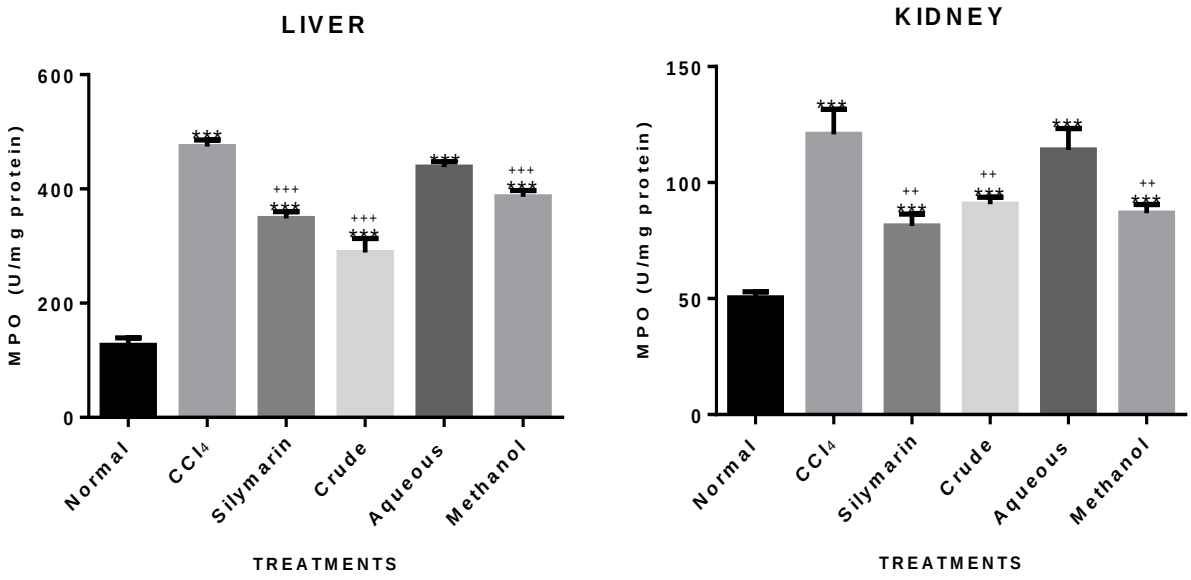

Figure 4.21 Effect of treatments on liver and kidney MPO levels. Each column represents a mean of 5 animals. ***p<0.001 against normal; $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 against CCl_4 group (MPO – Myeloperoxidase).

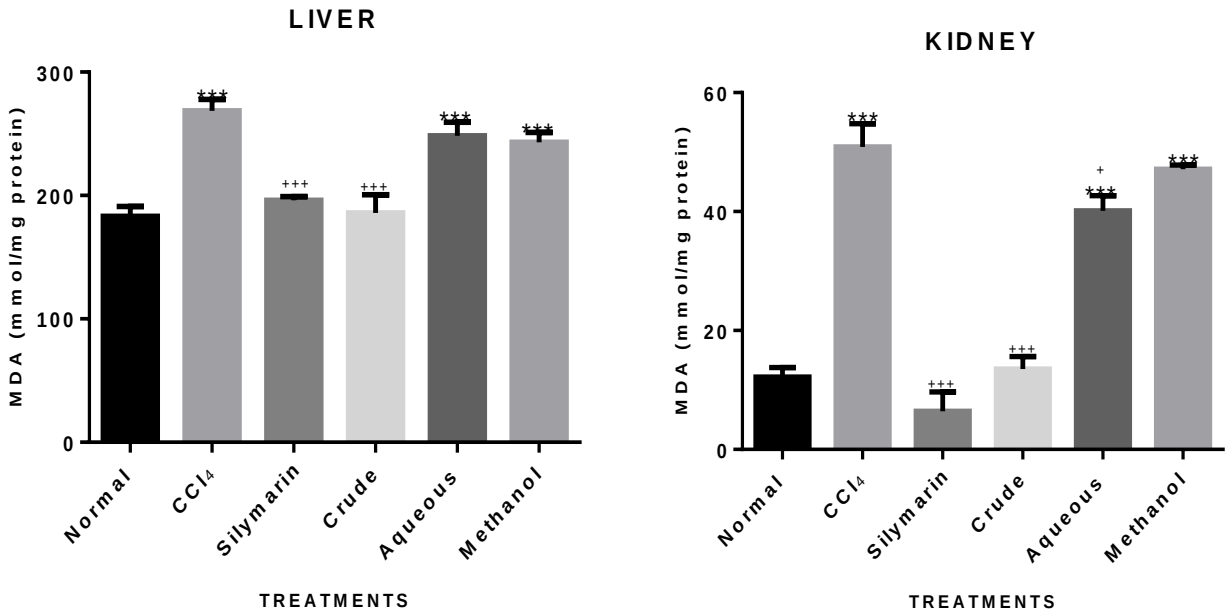

Figure 4.22 Effect of treatments on liver and kidney MDA levels. Each column represents a mean of 5 animals. ***p<0.001 against normal; +p<0.05, +++p<0.001 against CCl_4 group (MDA – Malondialdehyde).

CHAPTER FIVE

5.0 DISCUSSION

Aerobic organisms such as plants and animals need oxygen for energy metabolism, thus resulting in production of free radicals. Free radicals play an important role in the body. However, excessive generation of these free radicals could cause numerous damages including depletion of the immune system antioxidants, alteration to gene expression and eventually leading to the formation of abnormal proteins. Antioxidants are substance that can inhibit the formation of free radicals through scavenging mechanism (Hegde and Joshi, 2009). One method of determining the free radicals activity is the DPPH assay. It is based on the ability of 2, 2-diphenyl-1-picrylhydrazyl, a free radical to react with hydrogen donors such as phenols. DPPH assay is usually measured by an absorbance of 514 nm, which decreases with the scavenging of the free radicals (Jao and Ko, 2002). Activity of antioxidants were expressed in EC_{50}, which measures the concentration (mg/mL) of extracts that could inhibits or scavenge 50 % of DPPH radicals. The EC_{50} of crude extract of *A. conyzoides* was found to be 0.14 ± 0.01 µg/mL, which is almost the same as ascorbic acid (0.18 ± 0.06 µg/mL) (Table 4.1). In the same investigation as shown in Table 4.1, the aqueous fraction of *A. conyzoide*s was also seen to effectively inhibit DPPH radicals at a concentration of 0.20 ± 0.04 mg/ml. Methanol fraction reported a lower EC_{50} value of 3.56 ± 2.67 ($P < 0.001$ against Ascorbic acid), while ethyl acetate fractions recorded a weakest antioxidant property with EC_{50} value > 5 mg/mL. Phenolic compounds were present in the crude extract and all the fractions, with the highest TPC reported in the crude extract and lowest recorded in ethyl acetate fraction (Table 4.2). In this study, the total phenols present were suggested to be responsible for the antioxidant activity of *Ageratum conyzoides* crude extract and its fractions, as reported by Acheampong *et*

al., (2015). The various phenolic compound present suggested the antioxidant properties of the extracts, its ability to neutralize and scavenge free radicals. Acheampong *et al.* (2015), reported the antioxidant properties of hydroethanolic extract of *A. conyzoides* and suggested bioactive compounds responsible for such effect was phenol and flavonoids, hence it is suggested that the antioxidants activity of the extracts was partly due to the phenolic compounds present. This study suggests that *Ageratum conyzoides* possess antioxidant properties and this activity was predominantly observed in the crude extract.

The liver is an organ with multipurpose task; it plays a key role in the regulation of internal chemicals. Critical consequence occur when the liver is damaged by hepatotoxic agents. The kidney is also one major organ that help the body in detoxifying waste substances. In the present studies pre-administration of animals with *A. conyzoide* before inducing CCl_4 resulted in a significant protection against CCl_4 elevation of marker enzymes. Elevated serum enzymes and reduction in the levels of serum total proteins as well as alteration in several other parameters may be an indication of liver or kidney damage (Mansour and Mossa, 2010). Administration of CCl_4 to rats in the studies did not cause death of animal in any of the group, neither was there any effect on the body weight weights nor their general behaviour, however the weight of the liver increased significantly after treatment with CCl_4 (Table 4.8). In most cases, liver and kidney intoxicated with CCl_4 are expected to increase in weight due to its enlargement (Uemitsu, 1986). The enlargement of liver and kidney often occur after treatment with CCl_4 due to the infiltration of fatty acid and glycerol into the hepatocytes and nephron upon damage of the cell membrane. In this investigation, the weight of both organs (liver and kidney) were significantly affected by treatment with CCl_4. The cellular integrity and normal functioning of the liver was determined

by the concentration of ALT, AST, ALP and bilirubin in the blood serum (Gowda *et al.*, 2009). The levels of serum transaminases (ALT, AST and GGT; Figure 4.11) and bilirubin increased significantly in the group treated with CCl_4, thus the presence of CCl_4 was suggested to damage the liver causing these liver enzymes to leak into the blood serum (Rutherford and Chung, 2008). This agrees with results obtained by LeSage *et al.* (1999) in a similar investigation. Serum transaminases level of rat treated with crude extract and fractions after CCl_4 induction were observed to have significantly reduced. Crude extracts and fractions of *A. conyzoides* administration to the rats before intoxication was purposely to condition the hepatocytes to accelerate the regeneration of parenchymal cells, hence avoiding or decreasing the leaking of liver enzymes into serum through the protection of membrane. High liver protection (Figure 4.15) was seen in the group treated with the crude extract, thus significantly decreasing the level of enzymes markers into the circulating blood. The two fractions of *A. conyzoides* namely aqueous and methanolic fractions also demonstrated hepatoprotective effect against CCl_4, hence decreasing the level of serum transaminase (ALT, AST and GGT) and bilirubin in the blood serum as compared to the rats treated with only CCl_4. Preclinical treatment of animal with silymarin was observed to significantly decrease the level of serum marker enzymes in the blood serum (Figure 4.11-13).

Due to the high concentration of AST and ALT in hepatocytes, they are mostly described as liver enzymes, however, only ALT is marked specifically for liver function since it is produced specifically in the hepatocytes making it more specific to detect liver damage compared to AST, which could be present in liver, red blood cells and muscle. Muscle injury and blood haemolysis can result in increasing level of AST.

The half-life of AST is about 12 hours while the half-life of ALT is 60 hours, hence making serum levels of ALT and AST rise and fall in parallel (Ramaiah, 2007).

Level of gamma glutamyl transferase (GGT) was raised after CCl_4 administration, however it was decreased significantly after prophylactic treatment with crude extract and fractions (Figure 4.12). Impaired bile flow is detected by GGT and ALP. The presence of total serum bilirubin can also detect cholestasis in addition to the roles of GGT and ALP in determining hepatocellular damage (Ramaiah, 2007). In the experiment, the total bilirubin levels in the serum of rats treated with CCl_4 only increased significantly, however the levels decreased significantly when treated with the crude extracts and fractions of *Ageratum conyzoides* (Figure 4.12). Group treated with silymarin was also observed to have significantly low level of total bilirubin as compared to CCl_4 treated group.

Silymarin is a popular drug for patients known to have hepatotherapeutic and anti-fibrotic properties (Min *et al.,* 2007). Silymarin is also proven to have protective effect against genomic injury, decreasing tumour promoter activity, increasing hepatocyte protein synthesis and stabilizing mast cells. In many research, it has been proven to have a low bioavailability (Comoglio *et al*., 1995). The hepatoprotective potential demonstrated by the crude extract and hydroethanolic fraction at 250 mg/kg dose respectively were comparable to silymarin on CCl_4-induced liver injury rats. The altered level of high liver enzymes in the plasma were seen to return to the nearly normal profile after treatment with 250 mg/kg crude extract and hydroethanolic fraction of *A. conyzoides*.

Liver cirrhosis is characterized by abnormal changes in the liver lobular structure, occurrence of fibrosis and hepatic parenchyma lesion (Li and Crawford, 2004). Results

from histological findings proved that hydroethanolic crude extract of *A. conyzoides* positively affected the liver's structural recovery in CCl_4-induced liver cirrhosis rats (Figure 4-16, Table 4-9). No fibrosis with architectural distortion of the liver was significantly observed in the group treated with hydroethanolic fraction of the plant extract. There was indeed a remarkable reduction of fibrosis damage and a decrease of stellate infiltration in rats treated with both the plant crude extract and its isolated aqueous fraction. Hepatoprotective effect of *A. conyzoides* crude extract was greatly confirmed by the histological studies. Rats treated with CCl_4 had their liver sections showing necrosis, resulting in ballooning of the hepatocytes. 250 mg/kg dose of hydroethanolic fraction of *A. conyzoides* extract almost normalized the histo-architecture effect of liver much better than 250 mg/kg of methanolic fraction. The severe changes caused by CCl_4 in the liver of rats were eventually treated with the crude extract of *A. conyzoide* thus restoring the architectural structures of these organs. It was also observed to improve the liver's cellular integrity, as well as it functions by significantly decreasing the level of ALT, AST, ALP, GGT and bilirubin.

Cholesterol is described as a substance usually appearing as soft wax located in the blood stream of the body cells. It is mostly used in the formation of cell membranes and also in the production of hormones. Total cholesterol level depends on the balance between the rate of production by the body and absorption from diet. Elevated level of cholesterol signifies liver damage. In the studies, elevated levels of cholesterol were reversed to normal by treatment with crude extract of *A. conyzoide* and moderate reduction by its fractions (Figure 4-14). However, no significant change in triglyceride concentration was observed after extract treatment. Based on the reports of the study, hydroethanolic crude extract of *A. conyzoide* could be suggested to be a promising hepatoprotective agent against CCl_4 – induced damage rats

Furthermore, nephroprotective activities of crude extracts of *A. conyzoides* and its fractions were determined by observing the concentration level of creatinine and urea in the blood serum of rats treated with CCl_4 (Figure 4-13). Creatinine and urea are mostly considered as key markers in determining the normal function of kidney (Dharnidharka *et al*., 2002). Usually, increase in serum concentrations of these markers are possible indication for renal injury (Dharnidharka *et al*., 2002). In the studies, significant increase of 74.4 ± 0.51 µmol/L and 14.18 ± 0.15 mmol/L serum concentration of creatinine and urea respectively were observed in the CCl_4 only group as compared to the normal group. These alteration in the biochemical enzymes were corroborated by the histological finding which showed tubular hyalinization, glomerusclerosis, with interstitial inflammation of kidney in the rats intoxicated with only CCl_4 (Figure 4-17, Table 4-9). However, daily pre-treatment of rats with crude extract of *A. conyzoides* for 7 days confirmed the plant's nephroprotection against CCl_4 renal injury, with histology showing no inflammation or congestion and a significant decrease in creatinine and urea serum concentration (53.46 ± 0.646 µmol/L and 0.614 ± 0.116 mmol/L respectively). The two fractions were also seen to have moderate nephroprotective effect against CCl_4 renal injury in rat.

The mechanism for possible protection of *A. conyzoides* crude extract was suggested to be mediated by its antioxidant activities. This confirms the reports from literature reviews showing nephroprotective effects of medicinal plants mediated through their antioxidants properties due to the high levels of flavonoids and alkaloid concentrations present (Miller and Rice- Evans, 1997). According to Khan *et al*. (2010), saponins have also been recorded to protect the kidney and liver against CCl_4 intoxication. Hydroethanolic crude extract of *A. conyzoides* has also been reported to contain alkaloids, saponins, flavonoids and other active phytochemicals (Acheampong *et al*.,

2015). Based on the reported facts, it is possible the alkaloid, flavonoid and saponin phytochemicals present could be responsible for the nephroprotective effect of *A. conyzoides*. The antioxidants properties of *A. conyzoides* crude extract could play a key role in the nephroprotective property of the plant.

Cells of mammals are well equipped with antioxidant enzymes such as SOD, CAT, GST and GPx, which help prevent reactive oxygen species formation, as well as their damage effects. In the present investigation, significant changes were observed in SOD, CAT, GSH, MPO and MDA after treatment with CCl_4, thus suggesting high oxidative damage in the liver. SOD is involved in scavenging superoxide radicals to form hydrogen peroxide (H_2O_2), which eventually diminishes the toxic effect likely caused by free radicals. SOD is a sensitive index in liver injury, thus a reduction in SOD enzymatic activity could possibly suggest hepatocellular damage. SOD was reported by Rajkapoor *et al.* (2008) as an important enzyme in the enzymatic antioxidant defence system. Report from Palanivel *et al*. (2008) suggested that *P. aculeate* caused a significant increase of SOD activity in the hepatic cells and this resulted in a reduction of reactive free radical and thus oxidative injury to the liver was avoided. In the current study, enzyme activity of SOD was significantly reduced in both the liver (1.646 ± 0.138 U/mg) and kidney (1.590 ± 0.157 U/mg) group treated with only CCl_4 compared with normal group (4.037 ± 0.228 U/mg for liver and 3.736 ± 0.036 U/mg for kidney, Figure 4.18). However, treatment with the crude extract of *A. conyzoides* normalized the SOD enzyme activity, thus showing no significant difference in both organs when compared to normal group. Histological findings also confirm the absence of oxidative damage on these organs (Figure 4.16-17). The fractions also showed a significant change in SOD enzyme activity when compared to the CCl_4 induced group, however no significant change was observed in liver after

treating group with methanolic fraction of *A. conyzoides* when compared to CCl_4 group, oxidative damage on liver was reported for this group by histopathological findings.

CAT is another important enzymatic antioxidant widely spread in tissues of all animals with the liver and red cells having the highest activity. Hydrogen peroxide decomposition is catalysed by CAT and prevent the effect of highly reactive hydroxyl radicals on tissues (Rajkapoor *et al.*, 2008). Therefore, the accumulation of superoxide radical and hydrogen peroxide could result in deleterious effect of CAT, thus reducing its enzymatic activity and exposing the liver to oxidative damage. Administration of 250 mg/kg of crude extract and fractions of *A. conyzoides* increased the level of CAT as produced by the standard hepatoprotective drug, silymarin (Figure 4.19). Treatment with CCl_4 was observed having a significant reduction in CAT activity for both tissues (liver and kidney) with histological finding confirming tremendous damage to the cellular structures of the organs. In the liver, treatment with crude extract of *A. conyzoides* and fractions reported a significant increase in CAT enzymatic activity when compared to CCl_4 induced group, however in the kidney, only the crude extract recorded a significant increase in CAT activity.

Glutathione (GSH), a non – enzymatic biological antioxidant, mostly found in the liver also help in the scavenging of free radicals such as hydrogen peroxide, superoxide radicals and also maintain level of thiol protein in membrane. GSH also serve as a substrate for glutathione peroxidase (GPx) (Prakash *et al.*, 2001). Administration of crude extract of *A. conyzoides* resulted in significant ($P < 0.001$) increase GSH level, however decreased GSH level in CCl_4 only treated rats was associated with an enhanced lipid peroxidation (Figure 4.20).

MPO is a haem peroxidase, which is responsible for the formation of numerous ROS and also has a strong proinflammatory and pro-oxidative properties (Förstermann, 2008). The presence of MPO converts hydrogen peroxide to hypochlorous acid, in the presence of chlorine ion (Förstermann, 2008). In the study, rats intoxicated with CCl_4 were seen to have elevated levels of MPO activity (Figure 4.21), an index of hepatic infiltration (Belcastro *et al.,* 1996). Pre-treatment with *A. conyzoides* crude extract significantly reduced the level of hepatic MPO activity in both the kidney and liver induced by CCl_4, thus preventing neutrophils infiltration into both tissues. Agents such as curcumin and melatonin were also reported in previous studies to decrease hepatic MPO activity in CCl_4 intoxicated liver (Faghihzadeh *et al*., 2015).

The level of MDA is usually considered as a liver damage indicator; it's a reactive aldehyde which is mostly released during membrane phospholipid peroxidation. Elevated level of MDA was observed in the rats intoxicated with CCl_4, however treatment with *A. conyzoides* crude extract significantly reduced the level of MDA (Figure 4-22). Its free radical scavenging property was suggested to be the allotting factor for MDA level reduction (Wang *et al.,* 2009). Research reported by Kumar *et al.* (2011) also suggest a significant reduction of hepatic MDA levels in liver injury after treatment with *A. tricolor* and *A. caudatus.*

Phytochemicals play important roles in helping the body to fight against health problems. They prevent the formation of free radical chain by binding to some biomolecules, thus stabilizing the activity of free radicals formed and prevent damage within the body (Pham-Huy *et al.,* 2008). Table 4.3 of the present study reveals the presence of phytochemicals such as alkaloid, flavonoid, saponin, phenol, phytosterol and glycoside in the crude extract of *A. conyzoides.* Both aqueous and methanol fraction of the extract also reported presence of phytochemicals present.

Ethyl acetate recorded the absence of flavonoid, alkaloid and phenol, thus suggesting its poor scavenging potential against free radicals and hence excluded from further analyses during studies. High antioxidant property of the crude extract of *A. conyzoides* was attributed to the presence of alkaloid, flavonoids and phenol with both methanolic and hydroethanolic fractions showing moderate scavenging potential. The presence of these phytochemicals also suggested to be responsible for the antidysentreric, bacteriocide, antilithic, fever, rheumatism, headache and colic properties of *A. conyzoides* (Shekhar and Anju, 2017). The scavenging capability of flavonoid to neutralize free radical had also been reported by Edeoga *et al.*, (2005). Tannins, as also reported to have wound healing potential, as well as, inflaming mucous membrane (Okwu and Ekeke, 2003). Ahmad *et al.*, (2013), also reported the cytotoxic effect exhibited by saponins and its ability to inhibit growth of cell that makes them possess both anticancer and anti-inflammatory activities.

In this study, the antioxidant ability, hepatoprotective and nephron protect effect of the crude extract and fractions, as well as, it potential to scavenge free radicals was partly attributed to the presence of alkaloid, flavonoids and compounds containing phenol as reported by Acheampong *et al.*, (2015).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Data from this study suggested that crude ethanolic leaves extract of *A. conyzoides*, as well as, its hydroethanolic fractions had hepatoprotective and nephroprotective properties, thus increasing the level SOD, CAT and GSH activity after treatment with CCl_4 which had been recorded to cause damage to tissue after exposure. Phytochemicals such as alkaloid, phenolic compounds and flavonoids were suggested to partly contribute to the plant's hepatoprotective and nephroprotective effects.

The plants could therefore be considered as an alternative source for management of liver diseases usually caused by free radicals. Further investigation is needed to identify the most active components responsible for the hepatoprotective effects and also to unravel the mechanisms of actions of the actives principles.

6.2 RECOMMENDATIONS FOR FURTHER WORK

Further studies are needed to determine the bioavailability, pharmacodynamics, pharmacokinetics and other pharmacological evaluation of the plant extract and its fractions. The prevention mechanism of oxidative stress also applied on cell lines could also be investigated during further studies. Finally, potential active components responsible for the plants hepatoprotective and nephroprotective effects should be extensively studied, as well as, it mechanisms of actions.

REFERENCES

Adebayo, A., Tan, N., Akindahunsi, A., Zeng, G. and Zhang, Y. (2010). Anticancer and antiradical scavenging activity of *Ageratum conyzoides* L. (Asteraceae). *Pharmacognosy Magazine*, 6, 62-66.

Acheampong, F., Larbie, C., Appiah-Opong, R., Fareed, K.N.A. and Tuffour, I. (2015). In vitro antioxidant and anticancer properties of hydroethanolic extracts and fractions of *Ageratum conyzoides*. *European J Med Plants*, 7(4), 205-14.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A. and Anwar, F. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 337-352.

Ahmed, S., Rahman, A., Alam, A., Saleem, M., Athar, M. and Sultana, S. (2000). Evaluation of the efficacy of *Lawsonia alba* in the alleviation of carbon tetrachloride-induced oxidative stress. *Journal of Ethnopharmacology*, 69(2), 157-164.

Amat, N., Upur, H. and Blazeković, B. (2010). In vivo hepatoprotective activity of the aqueous extract of *Artemisia absinthium* L. against chemically and immunologically induced liver injuries in mice. *J Ethnopharmacol*., 131(2), 478-484.

Arthur, F.K.N., Woode, E., Terlabi, E.O., and Larbie, C. (2012). Evaluation of hepatoprotective effect of aqueous extract of *Annona muricata* (Linn.) leaf against carbon tetrachloride and acetaminophen induced liver damage. *Journal of Natural Pharmaceuticals*: 3 (1); 25 – 30.

Asadi-Samani, M., Kafash-Farkhad, N., Azimi, N., Fasihi, A., Alinia-Ahandani, E. and Rafieian-Kopaei, M. (2015). Medicinal plants with hepatoprotective activity in Iranian folk medicine. *Asian Pacific Journal of Tropical Biomedicine*, 5(2), 146-157.

Ayoola, G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia, E. and Atangbayila, T. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, 7, 1019-1024.

Bahirwani, R. and Reddy, K. R. (2014). Drug-induced liver injury due to cancer chemotherapeutic agents. *Seminars in Liver Disease*, 34, 162-171.

Bandaranayake, W. M. (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management*, 10(6), 421-452.

Bandyopadhyay, U., Das, D. and Banerjee, R. K. (1999). Reactive oxygen species: oxidative damage and pathogenesis. *Current Science*, 77(5), 658-666.

Belcastro, A. N., Arthur, G. D., Albisser, T. A. and Raj, D.A. (1996). Heart, liver, and skeletal muscle myeloperoxidase activity during exercise. *Journal of Applied Physiology*, 80(4), 1331-1335.

Bhawna, S. and Kumar, S. U. (2009). Hepatoprotective activity of some indigenous plants. *Int J Pharm Tech Res*, 4, 1330-1334.

Bhanwra, S. A., Singh, J., and Khosla, P. (2000). Effect of *Azadirachta Ondica* (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats. *Indian Journal of Physiology and Pharmacology*, 44(1), 64-68.

Blankson, A., Wiredu, E. K., Gyasi, R. K., Adjei, A. and Tettey, Y. (2005). Sero-prevalence of Hepatitis B and C viruses in cirrhosis of the liver in Accra, Ghana. *Ghana Medical Journal*, 39(4), 132.

Boadu Augustine and Alex Asase. (2017) Documentation of Herbal Medicines Used for the Treatment and Management of Human Diseases by Some Communities in Southern Ghana. *Evidence-Based Complementary and Alternative Medicine*, 85 (4) 12-15.

Bosch, F. X., Ribes, J., Díaz, M. and Cléries, R. (2004). Primary liver cancer: worldwide incidence and trends. *Gastroenterology*, 127(5), S5-S16.

Chuihua, K., Tao, X. and Fei, H. (1998). Allelopathy of *Ageratum conyzoides*. releasing mode and activity of main allelochemicals. *Chinese Journal of Applied Ecology*, 9(3), 257-260.

Comoglio, A., Tomasi, A., Malandrino, S., Poli, G. and Albano, E. (1995). Scavenging effect of silipide, a new silybin-phospholipid complex, on ethanol-derived free radicals. *Biochemical Pharmacology*, 50(8), 1313-1316.

Daily, E. B. and Aquilante, C. L. (2009). Cytochrome P450 2C8 pharmacogenetics: a review of clinical studies. *Pharmacogenomics,* 10(9), 1489-1510.

Dalziel, J. M. (1937). The useful plants of west tropical Africa. *The Crown Agents for the Colonies,* London. pp. 52-560.

Dharnidharka, V.R., Kwon, C. and Stevens, G. (2002). Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *American Journal of Kidney Diseases*, 40(2), 221-226.

Dongdem, J. T., Kampo, S., Soyiri, I. N., Asebga, P. N., Ziem, J. B., and Sagoe, K. (2012). Prevalence of hepatitis B virus infection among blood donors at the Tamale Teaching Hospital, Ghana (2009). *BMC Research Notes*, 5(1), 115.

Doughari, J. H. (2012). Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In Phytochemicals-A global perspective of their role in nutrition and health. *InTech* 1, 20-30.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology,* 4(7), 685-688.

Edington, G. M. (1957). Observations on hepatic disease in the Gold Coast: with special reference to cirrhosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 51(1), 48IN351-50IN455.

Edmonds, J. S. and Francesconi, K. A. (1993). Arsenic in seafoods: human health aspects and regulations. *Marine Pollution Bulletin*, 26(12), 665-674.

Ellman, M. (1959). A spectrophotometric method for determination of reduced glutathione in tissues. *Analyt Biochem,* 74, 214-226.

El-Sayed, E.M., Fouda, E.E., Mansour, A.M. and Elazab, A.H.(2015). Protective effect of lycopene against carbon tetrachloride-induced hepatic damage in rats. *International Journal of Pharma Sciences,* 5, 875-881.

Evans, W. (2009). Trease and evans pharmacognosy 16th ed. Saunders: Elsevier Ltd. China.

Faghihzadeh, F., Hekmatdoost, A. and Adibi, P. (2015). Resveratrol and liver: A systematic review. *Journal of Research in Medical Sciences,* 20(8), 797.

Förstermann, U. (2008). Oxidative stress in vascular disease: causes, defence mechanisms and potential therapies. *Nature Reviews; Cardiology*, 5(6), 338.

Friedman, S.L. (2000). Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *Journal of Biological Chemistry*, 275(4), 2247-2250.

Garcia-Tsao, G., and Lim, J. (2009). Management and treatment of patients with cirrhosis and portal hypertension: recommendations from the Department of Veterans Affairs Hepatitis C Resource Center Program and the National Hepatitis C Program. *The American Journal of Gastroenterology*, 104(7), 1802.

Ghasemi, K., Ghasemi, Y. and Ebrahimzadeh, M. A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Sciences*, 22: 277-281.

Gill, R. Q. and Sterling, R. K. (2001). Acute liver failure. *Journal of Clinical Gastroenterology*, 33(3), 191-198.

Gowda, S., Desai, P. B., Hull, V. V., Math, A. A. K., Vernekar, S. N. and Kulkarni, S. S. (2009). A review on laboratory liver function tests. *The Pan African Medical Journal*, 3, 10-15.

Govindarajan, R., Rastogi, S., Vijayakumar, M., Shirwaikar, A., Rawat, A. K. S., Mehrotra, S. and Pushpangadan, P. (2003). Studies on the antioxidant activities of *Desmodium gangeticum*. *Biol. Pharm. Bull.,* 26**:** 1424-1427.

Granato, D., Branco, G.F., Nazzaro, F., Cruz, A.G. and Faria, J.A. (2010). Functional foods and nondairy probiotic food development: trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety*, 9(3), 292-302.

Gutierrez-Reyes, G., Gutierrez-Ruiz, M. C. and Kershenobich, D. (2007). Liver fibrosis and chronic viral hepatitis. *Archives of Medical Research*, 38(6), 644-651.

Gyamfi, M. A., Yonamine, M. and Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *General Pharmacology*, 32(6), 661-667.

Hancock, J. T., Desikan, R. and Neill, S. J. (2001). Role of reactive oxygen species in cell signalling pathways. *Biochem Soc Trans*. 29(2), 345-349.

Hegde, K. and Joshi, A. B. (2009). Hepatoprotective effect of *Carissa carandas* Linn root extract against CCl_4 and paracetamol induced hepatic oxidative stress. *Indian Journal of Experimental Biology*, 47, 660.

Ita S.O., Etim O.E., Ben E.E. and Ekpo O.F. (2009). Haemato-poietic properties of ethanolic leaf extract of *Ageratum conyzoides* (Goat weed) in albino rats. *Nigerian Journal of Physiology and Science*, 22, 83-87.

Iwu, M. W., Duncan, A. R. and Okunji, C. O. (1999). New antimicrobials of plant origin. Perspectives on new crops and new uses. *ASHS Press, Alexandria*, VA, 457-462.

Jaeschke, H., Knight, T. R. and Bajt, M. L. (2003). The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. *Toxicology letters*, 144(3), 279-288.

Jagetia, G.C., Shirwaikar, A., Rao, S.K. and Bhilegaonkar, P.M. (2003). Evaluation of the radioprotective effect of *Ageratum conyzoides Linn*. extract in mice exposed to different doses of gamma radiation. *Journal of Pharmacy and Pharmacology*, 55, 1151-1158.

Jao, C.L. and Ko, W.C. (2002). 2, 2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. *Fisheries Science*, 68(2), 430-435.

Jemal, A., Murray, T., Ward, E., Samuels, A., Tiwari, R. C., Ghafoor, A. and Thun, M. J. (2005). Cancer statistics. *A Cancer Journal for Clinicians*, 55(1), 10-30.

Kamboj, A. and Saluja, A., (2008). *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. *International Journal of Green Pharmacy*, 2(2), 59.

Kanbur, M., Eraslan, G., Sarıca, Z. S. and Aslan, Ö. (2011). The effects of evening primrose oil on lipid peroxidation induced by subacute aflatoxin exposure in mice. *Food and Chemical Toxicology*, 49(9), 1960-1964.

Kaufman, B., Gandhi, S. A., Louie, E., Rizzi, R., and Illei, P. (1997). Herpes simplex virus hepatitis: case report and review. *Clinical Infectious Diseases*, 24(3), 334-338.

Khan, R.A., Khan, M.R. and Sahreen, S. (2010). Evaluation of *Launaea procumbens* used in renal disorders: a rat model. *Journal of Ethnopharmacology*, 128(2), 452-461.

Kim, Y., You, Y., Yoon, H.G., Lee, Y.H., Kim, K., Lee, J., Kim, M.S., Kim, J.C. and Jun, W. (2014). Hepatoprotective effects of fermented *Curcuma longa* L. on carbon tetrachloride-induced oxidative stress in rats. *Food Chemistry*, 151, 148-153.

Kumar, A., Tantry, B.A., Rahiman, S. and Gupta, U. (2011) Comparative study of antimicrobial activity and phytochemical analysis of methanolic and aqueous extracts of the fruit of *Emblica officinalis* against pathogenic bacteria. *Journal of Traditional Chinese Medicine*, 31, 246-250.

Kumar, R. P., Shammy, J., Nitin, G., and Rinu, R. (2014). An inside review of *Amaranthus spinosus* Linn: a potential medicinal plant of India. *International Journal of Research in Pharmacy and Chemistry*, 4(3), 643-653.

Landy, D. (1977). Culture, disease and healing: Studies in Medical Anthropology. Macmillan, New York. p 14.

Leal, L. K. A. M., Ferreira, A. A. G., Bezerra, G. A., Matos, F. J. A. and Viana, G. S. B. (2000). Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: a comparative study. *Journal of Ethnopharmacology*, 70(2), 151-159.

LeSage, G. D., Benedetti, A., Glaser, S., Marucci, L., Tretjak, Z., Caligiuri, A. and Lasater, J. (1999). Acute carbon tetrachloride feeding selectively damages large, but not small, cholangiocytes from normal rat liver. *Hepatology*, 29(2), 307-319.

Li, M.K. and Crawford, J.M. (2004). The pathology of cholestasis. In Seminars in Liver Disease. *Thieme Medical Publishers, Inc*., 24, (01), 21-42.

Lin, Y., Shi, R., Wang, X., and Shen, H. M. (2008). Luteolin, a flavonoid with potential for cancer prevention and therapy. *Current Cancer Drug Targets*, 8(7), 634-646.

Lim, Y. S., & Kim, W. R. (2008). The global impact of hepatic fibrosis and end-stage liver disease. *Clinics in Liver Disease*, 12(4), 733-746.

Liston, H. L., Markowitz, J. S., and DeVane, C. L. (2001). Drug glucuronidation in clinical psychopharmacology. *Journal of Clinical Psychopharmacology*, 21(5), 500-515.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. (1951). Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.*, 193, 265-275.

Maheswari, C., Maryammal, R. and Venkatanarayanan, R. (2008). Hepatoprotective activity of *Orthosiphon stamineus* on liver damage caused by paracetamol in rats. *Jordan J Biol Sci*, 1(3), 105-108.

Manov, I., Motanis, H., Frumin, I. and Iancu, T. C. (2006). Hepatotoxicity of anti-inflammatory and analgesic drugs: ultrastructural aspects. *Acta Pharmacologica Sinica*, 27(3), 259-272.

Mansour, S.A. and Mossa, A.T.H. (2010). Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorophyll and the antioxidant role of zinc. *Pesticide Biochemistry and Physiology*, 96(1), pp.14-23.

Marques Neto, J. F., Costallat, L. T. L., Fernandes, S. R. M., Napoli, M. D. M. D., & Samara, A. M. (1988). Efeitos do *Ageratum conyzoides*, Linèe no tratamento da artrose. *Rev. Bras. Reumatol*, 28(4), 109-14.

Miller, N.J. and Rice-Evans, C.A. (1997). The relative contribution of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem.*, 60, 331 – 337.

Min, K., Yoon, W.K., Kim, S.K. and Kim, B.H. (2007). Immunosuppressive effect of silibinin in experimental autoimmune encephalomyelitis. *Arch Pharm Res*., 30(10), 1265-72.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*., 25, 247(10), 3170-3175.

Muchuweti, M., Kativu, E., Mupure, C., Chidewe, C., Ndhlala, A. and Benhura, M. (2007). Phenolic composition and antioxidant properties of some spices. *American Journal of Food Technology*, 2: 414-420.

Murphy, K. and Weaver, C. (2016). Janeway's Immunobiology. *Garland Science*.

Murray, C. J. and Lopez, A. D. (1996). Global health statistics: a compendium of incidence prevalence and mortality estimates for over 200 conditions. Harvard School of Public Health. (Available at http://www.who.int/iris/handle/10665/41848)

Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J. and Gunsalus, I. C. (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics and Genomics*, 6(1), 1-42.

Neuwinger, H. D. (2000). African traditional medicine: a dictionary of plant use and applications with supplement: search system for diseases. Medpharm Scientific Publishers, Stuttgart, Germany. pp 7-10.

Nishigaki, I., Hagihara, M., Tsunekawa, H., Maseki, M. and Yagi, K. (1981). Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. *Biochemical Medicine*, 25(3), 373-378.

Nogueira J.H., Gonçalez E., Galleti S.R., Facanali R., Marques M.O. and Felício J.D. (2010). *Ageratum conyzoides* essential oil as aflatoxin suppressor of *Aspergillus flavus*. *International Journal of Food Microbiology*, 137, 55-60.

Nour, A.M., Khalid, S.A., Kaiser, M., Brun, R., Abdalla, W.l.E. and Schmidt, T.J. (2010). The antiprotozoal activity of methylated flavonoids from *Ageratum conyzoides* L. *Journal of Ethnopharmacology*, 129, 127-130.

Nyunaï, N., Manguelle-Dicoum, A., Njifutié N., Abdennebi E. and Gerard C. (2010). Antihyperglycaemic effect of *Ageratum conyzoides* L. fractions in normoglycemic and diabetic male Wistar rats. *International Journal of Biomedical and Pharmaceutical Sciences*, 4, 38-42.

Okwu, D. and Ekeke, O. (2003). Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. *Global Journal of Pure and Applied Sciences* 9, 235-238.

Opoku, A.R., Ndlovu, I. M., Terblanche, S.E. and Hutchings, A.H. (2007). In vivo hepatoprotective effects of *Rhoicissus tridentata* subsp. cuneifolia, a traditional Zulu medicinal plant, against CCl_4- induced acute liver injury in rats. *South African Journal of Botany*, 73, 372-377.

Orhan, D. D., Orhan, N., Ergun, E. and Ergun, F. (2007). Hepatoprotective effect of *Vitis vinifera* L. leaves on carbon tetrachloride-induced acute liver damage in rats. *Journal of Ethnopharmacology*, 112(1), 145-151.

Palanivel, M.G., Rajkapoor, B., Kumar, R.S., Einstein, J.W., Kumar, E.P., Kumar, M.R., Kavitha, K., Kumar, M.P. and Jayakar, B. (2008). Hepatoprotective and antioxidant effect of *Pisonia aculeata L*. against CCl_4- induced hepatic damage in rats. *Scientia Pharmaceutica*, 76, 203-215.

Parola, M., Leonarduzzi, G., Biasi, F., Albano, E., Biocca, M. E., Poli, G. and Dianzani, M. U. (1992). Vitamin E dietary supplementation protects against carbon tetrachloride—induced chronic liver damage and cirrhosis. *Hepatology*, 16(4), 1014-1021.

Parkin, D. M., Bray, F., Ferlay, J. and Pisani, P. (2001). Estimating the world cancer burden: Globocan 2000. *International Journal of Cancer*, 94(2), 153-156.

Patel, T., Roberts, L. R., Jones, B. A. and Gores, G. J. (1998). Dysregulation of apoptosis as a mechanism of liver disease: an overview. In Seminars in liver disease *Thieme Medical Publishers*, 18(2), 105-114.

Pham-Huy, L.A., He, H. and Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science: IJBS*, 4(2), 89.

Pirmohamed, M., Breckenridge, A. M., Kitteringham, N. R. and Park, B. K. (1998). Fortnightly review: adverse drug reactions. *BMJ: British Medical Journal*, 316(7140), 1295.

Porchezhian, E. and Ansari, S.H. (2005). Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. *Phytomedicine*, 12, 62-64.

Prakash, J., Gupta, S.K and Singh N. (2001). Chemopreventive activity of *Withania somnifera* in experimentally induced fibro sarcoma tumors in Swiss albino rats. *Phytother Res*.; 15: 200–204

Rajkapoor, B., Venugopal, Y., Anbu, J., Harikrishnan, N., Gobinath, M. and Ravichandran, V. (2008). Protective effect of *Phyllanthus polyphyllus* on acetaminophen induced hepatotoxicity in rats. *Pak J Pharm Sci*, 21(1), 57-62.

Ramaiah, S.K. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*, 45(9), 1551-1557.

Rice-Evans, C., Miller, N. and Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2(4), 152-159.

Rumack, B. H. (2004). Acetaminophen misconceptions. *Hepatology*, 40(1), 10-15.

Rutherford, A. and Chung, R. T. (2008). Acute liver failure: mechanisms of hepatocyte injury and regeneration. *Thieme Medical Publishers*, 28(2), 167-174.

Sarich, T. C., Adams, S. P., Petricca, G. and Wright, J. M. (1999). Inhibition of isoniazid-induced hepatotoxicity in rabbits by pre-treatment with an amidase inhibitor. *Journal of Pharmacology and Experimental Therapeutics*, 289(2), 695-702.

Schuppan, D., Jia, J. D., Brinkhaus, B. and Hahn, E. G. (1999). Herbal products for liver diseases: a therapeutic challenge for the new millennium. *Hepatology*, 30(4), 1099-1104

Şenoğlu, M., Kurutaş, E. B., Altun, I., Yalçın, A. T. L. I. and Nacitarhan, V. (2009). Myeloperoxidase activities in polymorphonuclear leukocytes in lumbar disc disease. *Journal of Neurological Sciences* 26(3), 292-297.

Shekhar, T.C. and Anju, G. (2017). A comprehensive review on *Ageratum conyzoides* Linn. (Goat weed). *International Journal of Pharmaceutical and Phytopharmacological Research*, 1(6), 391-395.

Shirwaikar, A., Bhilegaonkar, P., Malini, S. and Sharath Kumar, J. (2003). The gastroprotective activity of the ethanol extract of Ageratum conyzoides. *Journal of Ethnopharmacology*, 86, 117 121.

Shoeb, M., Jaspars, M., MacManus, S. M., Celik, S., Nahar, L., Kong-Thoo-Lin, P. and Sarker, S. D. (2007). Anti-colon cancer potential of phenolic compounds from the aerial parts of *Centaurea gigantea* (Asteraceae). *Journal of Natural Medicines*, 61(2), 164.

Singh, C.B., Devi W.R., Marina, A., Devi W.I., Swapana N. and Singh C.B. (2013). Ethnobotany, phytochemistry and pharmacology of *Ageratum conyzoides* Linn (Asteraceae). *Medicinal Plants Research*, 7(8), 371-385.

Si-Tayeb, K., Lemaigre, F.P. and Duncan, S.A., (2010). Organogenesis and development of the liver. *Developmental Cell*, 18, 175-189.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, 47(2), 389-394.

Sluzewska, A., Rybakowski, J., Bosmans, E., Sobieska, M., Berghmans, R., Maes, M. and Wiktorowicz, K. (1996). Indicators of immune activation in major depression. *Psychiatry Research*, 64(3), 161-167.

Stellon, A. J., Davies, A., Compston, J. and Williams, R. (1985). Osteoporosis in chronic cholestatic liver disease. *An International Journal of Medicine*, 57(2), 783-790.

Stringer, M. D. (2014). Anatomy and Development of the Liver. *In Diseases of the Liver in Children: Evaluation and Management.* Murray, K.F. and Horslen, S. (Eds). Springer, New York. pp 3-21.

Strobel, G. and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*, 67(4), 491-502.

Swarbrick, J.T. (1997). Weeds of the Pacific Islands. *Technical Paper-South Pacific Commission,* Issue 209.

Targhetta, C., Cabras, M. G., Mamusa, A. M., Mascia, G., and Angelucci, E. (2008). Hepatitis B virus-related liver disease in isolated anti-hepatitis B-core positive lymphoma patients receiving chemo-or chemo-immune therapy. *Haematologica,* 93(6), 951-952.

Timbrell, J. A. (2009). Principles of Biochemical Toxicology. 4th Edition. Informa

Healthcare, NY, USA. pp 308 – 327.

Uemitsu, N. (1986). Inhalation pharmacokinetics of carbon tetrachloride in rats based on arterial blood: inhaled air concentration ratios. *Toxicology and Applied Pharmacology*, 83(1), 20-29.

Wang, A.N., Yi, X.W., Yu, H.F., Dong, B. and Qiao, S.Y. (2009). Free radical scavenging activity of *Lactobacillus fermentum* in vitro and its antioxidative effect on growing–finishing pigs. *Journal of Applied Microbiology*, 107(4), 1140-1148.

Wasley, A., Gallagher, K. M. and Grytdal, S. (2008). Surveillance for acute viral hepatitis, United States, 2006. *MMWR Surveill Summ.,* 21, 57(2),1-24

Weber, L.W.D., Boll, M. and Stampf, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology*, 33, 105-136.

Wood, T. F., Rose, D. M., Chung, M., Allegra, D. P., Foshag, L. J. and Bilchik, A. J. (2000). Radiofrequency ablation of 231 unresectable hepatic tumors: indications, limitations, and complications. *Annals of Surgical Oncology*, 7(8), 593-600.

World Health Organization. (2005). WHO global atlas of traditional, complementary and alternative medicine (Vol. 2). World Health Organization.

World Health Organization, 2009. Global health risks: mortality and burden of disease attributable to selected major risks. World Health Organization.

Yoshida, T., Abe, K., Ikeda, T., Matsushita, T., Wake, K., Sato, T. and Inoue, H. (2007). Inhibitory effect of glycyrrhizin on lipopolysaccharide and d-galactosamine-induced mouse liver injury. *European Journal of Pharmacology*, 576(1), 136-142.

Yoshizawa, F., Kimball, S. R., Vary, T. C. and Jefferson, L. S. (1998). Effect of dietary protein on translation initiation in rat skeletal muscle and liver. *American Journal of Physiology*, 275(5), 814-820.