AMELIORATIVE EFFECT OF *n*-BUTANOL FRACTION OF METHANOL EXTRACTOF *FICUS GLUMOSA* LEAVES AGAINST CCl_4-INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN *ALBINO* RATS

BY

Michael Sunday ABU, BSc. (ABU) 2008
P13SCBC8010

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE DEGREE (M.Sc.) IN BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY
ZARIA-NIGERIA

NOVEMBER, 2016.

DECLARATION

I declare that the work in this dissertation entitled "**Ameliorative Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves against CCl_4 Induced Hepatotoxicity and Oxidative Stress in *Albino* Rats**" was performed by me in the Department of Biochemistry, Ahmadu Bello University Zaria under the supervision of Prof. Sani Ibrahim and Prof. Anigo Kola Mathew. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project was previously presented for another degree or diploma at any university.

Michael Sunday ABU ________________ ________________
(P13SCBC8010) Signature Date

CERTIFICATION

This dissertation entitled "**Ameliorative Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves against CCl_4 Induced Hepatotoxicity and Oxidative Stress in *Albino* Rats**" by Michael Sunday ABU meets the regulations governing the award of the degree of M.Sc. Biochemistry of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

ProfessorS. Ibrahim ____________ ____________

Chairman, Supervisory Committee (Signature) (Date)

Professor K.M Anigo ____________ ____________

Member, Supervisory Committee (Signature) (Date)

ProfessorM. N. Shuaibu ____________ ____________

Head of Department (Signature) (Date)

Professor K. Bala ____________ ____________

Dean, School of Postgraduate Studies (Signature) (Date)

DEDICATION

This work is dedicated to God Almighty who bestowed his abundant mercy upon me before and during the period of this work.

ACKNOWLEDGEMENT

Firstly, my honest gratitude goes to God Almighty for making this program a reality.

My unreserved appreciation to the team of highly proficient respected academia, Professors, Sani Ibrahim and Anigo Kola Mathew for their supervisory role, advice and contributions in making sure that this work was a huge success. Sir, you are my academic role models!

I thank the able Head of Biochemistry Department (ABU, Zaria), Professor M.N Shuaibu for his management role in making the environment inspiring for learning biochemistry and his profound encouragement to student.

My profound gratitude goes to all the lecturers of Biochemistry Department (ABU, Zaria) for impacting adequate knowledge on me to be able to carry out this work proficiently. I am proud of you all sir!

How can I forget the great team of laboratory technicians, Mr. Apeh (Biochemistry Dept., ABU, Zaria), Mr. Olu and Mr. James (Chempath, ABUTH, Zaria), the entire crew of Animal House (Pharmacology Dept., ABU, Zaria), Mr. Bamidele (Human Anatomy Dept., ABU, Zaria), Mallam Kabiru (Pharmacognosis Dept., ABU, Zaria) and Mallam Muhammed (NARICT, Zaria). You are all wonderful people!

To all my friends; roommates, course mates and those outside the academic environment, I say thank you all for what you have done for me especially during the period of this work.

I thank the Dean, School of Post Graduate Studies and the Dean, Faculty of Sciences, Ahmadu Bello University, Zaria for providing an atmosphere that made it possible for me to finish this study.

Finallyand most importantly,my unquantifiable gratitude goes to my family members, most especially my parents Mr Abu Ola Fabian and Mrs Abu Margret, my siblings and my uncle Dr Abu Agbo Cletus for their distinct roles in making this program a reality.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

ABC	ATP-binding Cassette
ADH	Antidiuretic Hormone
ALPAlkaline phosphatase	
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BCRP	Breast Cancer Resistance Protein
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toluene
DPPH	1,1-Dipenyl-2-picrylhydrazyl
GPx	Glutathione Peroxidase
GSH	Glutathione Reduced
HDL	High Density Lipoprotein
HNE	Hydroxynonenal
IGFR	Insulin-like Growth Receptor
LDL	Low Density Lipoprotein
MDA	Malondialdehyde
MMP-2	Metalloproteinase-2
MRP	Multi-drug Resistance-associated Proteins
NADPH	Nicotinamide Adenine dinucleotide Phosphate Reduced
NAPQIN-acetyl-p-benzo-quinoneimine	
NF-kB	Nuclear Factor kappa B-cells
NTCP	Sodium Taurocholate Co-transporting Polypeptide
OATP	Organic Anion-Transporting Polypeptide

P-gp	Polyglycoprotein
PSC	Primary Sclerosing Cholangitis
PUFAs	Polyunsaturated Fatty Acids
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TAG	Triacylglyerol
TBA	Thiobarbituric Acid
TC	Total Cholesterol
VEGF	Vascular Endothelial Growth Factor

ABSTRACT

Essential organ like liver that plays crucial roles in transforming and clearing chemical agents ingested into the body needs to be investigated for wide spectrum of protective and ameliorative therapeutic agents that are readily available and affordable. In this study, crude methanol extract of *Ficus glumosa* leaves was fractionated using solvent-to-solvent partition method in order to assess the ameliorative property of the fraction with the most active antioxidant potency against CCl_4-induced hepatotoxicity. The *n*-butanol fraction was then selected for the treatment of liver injury induced by CCl_4in*albino* rats based on the preliminary phytochemical and free radical scavenging activity assessment. Rats were induced once weekly with $CCl_4$and treated with three different graded doses (100 mg/kg/bw, 300 mg/kg/bw and 500 mg/kg/bw) of *n*-butanol fraction extract of *Ficus glumosa* leaves and 100 mg/kg/bw silymarin for 21 days. The crude methanol extract of *Ficus glumosa* leaves showed presence of flavonoids, saponnins, tannins, and alkaloids. On the other hand, *n*-butanol fraction showed higher concentrations of total phenol and flavonoids [9.76±0.63 (mg/100g) Gallic Acid Equivalent and 5.27±0.23 (mg/100g) Quercetin Equivalent respectively] as compared to aqueous, *n*-hexane and ethylacetate fractions, while the aqueous residue presented the highest concentration of ascorbic acid (0.387±0.08 mg/ml) expressed in terms of L-Ascorbic Acid Equivalent. However,the *n*-butanol fraction exhibited the lowest IC_{50} value of 0.41±0.07 mg/ml,while the IC_{50} values of aqueous residue, ethylacetate and *n*-hexane fraction were in the order of (0.71±0.05 mg/ml) < (0.93±0.13 mg/ml) < (2.77±0.49 mg/ml) respectively. Meanwhile the Median Lethal Dose (LD_{50}) of *n*-butanol fraction as determined was >5000 mg/kg body weight.Consequently, the *n*-butanol fraction significantly ($P<0.05$) reduced liver marker enzymes [Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)], bilirubin, malondialdehyde (MDA) with concomitant increase in serum proteins, albumin, catalase, (CAT), glutathione

peroxidase (GPx) and superoxide dismutase (SOD) levels in a dose dependent manner in treated animals as compared to the untreated rats. Likewise, the *n*-butanol fraction significantly (P<0.05) decreased the elevated levels of creatinine, urea, electrolytes (Na^+, K^+, Cl^+) and restored the deplorable antioxidant status in the kidney in a dose dependent manner as compared to the untreated rats. Furthermore, there was a significant (P<0.05) reduction of the total cholesterol and white blood cell (WBC) with corresponding increase in high density lipoprotein (HDLc), packed cell volume (PCV) and haemoglobin (Hb) levels in rats treated with *n*-butanol fraction comparable to the effect of silymarin standard drug. The results may suggest that *n*-butanol fraction ofmethanol extract of *Ficus glumosa* leaves could possibly ameliorate oxidative damage induced by CCl_4 in both liver and kidney.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The use of plants in traditional medicine has enhanced the potentials to achieve most objectives of primary healthcare delivery, not only in Africa but also, virtually in most countries of the world (Elujoba *et al.,* 2005).Plants have the ability to synthesise a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Lai and Roy, 2004; Tapsell *et al.*, 2006). Chemical compounds obtain from plants often mediate their therapeutic effects on the human body through processes that are identical to those already well understood mechanisms in conventional drugs (Tapsell *et al.*, 2006).It is generally assumed that the active constituents contributing to the medicinal values of plants are the phytochemicals, minerals and vitamins which usually confer on them some useful preventive or curative mechanisms against cellular deformation. Consequently, Jamuna *et al.* (2014) demonstrated that the presence of phytochemicals such as alkaloids, phenolics, flavonoids, tannins, saponins and ascorbic acid is clearly responsible for the prominent antioxidant properties of *Hypochaeris radicata*.

In view of the presumed benefits of compounds present in plants, ethno-botany which is the study of traditional use of plants by man is recognized as an effective way to discover future medicines and currently, many of the pharmaceutical drugs such as aspirin, quinine, digitalis and opium available to physicians have a long history of use as herbal remedies in ethnomedicinal practice (Fabricant and Farnworth, 2001). The use of herbs to treat disease/disorders is almost common throughout non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceutical formulations (Edgar *et al.*, 2002). In India, the herbal remedy is so popular that the government has created a separate department called Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) under the Ministry of Health and Family Welfare and the National Medicinal Plants Board to deal with the herbal medical system in the country (Kala *et al.*, 2007) whereas it is obvious that the situation of herbal medical practice in Nigeria remained unattended to by either the government or any organised body.

Treatment and management of liver disorders seems to be a major challenge to modern medicine and in the absence of reliable liver protective drugs in allopathic medical practices, herb becomes key in the management of various liver disorders (Buraimoh *et al.*, 2011). Consequently, due to numerous protective tendency of herbal preparations which are accessible and do not require laborious pharmaceutical synthesis and processes, many plants have been used to minimize hepatic and other cellular damages induced by different chemical agents (Adaeze *et al*., 2015).This study furthermore investigated the ameliorative effects of *Ficus glumosa* against hepatotoxicity and oxidative stress induced by CCl_4 in*albino* rats.

1.2 Statement of Research Problem

It is imperative to understand that with the present day human activities, so many chemical additives and pollutants are now found in the air, water and food that calls for proactive and active measures in order to avert some health threatening potentials of such agents to human beings and economic animals (Akharaiyi *et al.,* 2012). Therefore, essential organ like liver that plays crucial roles in transforming and clearing chemical agents ingested into the body knowingly or unknowingly which may sometimes in the process becomes susceptible to the toxicity from these agents (Abiodun *et al.*, 2014), needs to be investigated for wide spectrum of protective and ameliorative therapeutic agents that are readily available and affordable.

1.3 Justification

The study of African medicinal plants has not in the past been taken as seriously, or documented as fully, as Indian and Chinese traditional medicines such that, theover 5000 plants known to be used for medicinal purposes in Africa, only a few have been studied (Abiodun*et al.*, 2014).Furthermore, plants are important sources of drugs for the treatment of several ailments such as inflammation, jaundice, oedema, hypertension among others owe to the therapeutic activity of their diverse secondary metabolites and more so, they are readily available, affordable and easily acceptable within our society (Ezeonwu and Dahiru, 2013).

1.4 Aim

The aim of this study is to investigate the ameliorative effect of *n*-butanol fraction of crude methanol extract of *Ficus glumosa* leaves on the liver damage and oxidative stress induced by CCl_4 in*albino* rats.

1.4.1 Specific Objectives

i. To assess some key phytochemical constituents and antioxidant potency of different solvent fractions of methanol extract of *Ficus glumosa* leaves.

ii. To assess ameliorative property of methanol extract fraction of *Ficus glumosa* leaves with most active antioxidant potency on CCl_4 damaged liver and kidney of *albino* rats.

iii. To assess the effect of the most active fraction of methanol extract of *Ficus glumosa* leaves on some serum lipid profile and some haematological parameters of the experimental*albino* rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Ficus Glumosa*

2.1.1 Scientific Classification

Family: *Moraceae*

Genus: *Ficus*

Species: *Glumosa*

Botanical name: *Ficus glumosa*

Plate I: (a) *Ficus Glumosa* Leaves (Snapshot) (b) *Ficus Glumosa;* whole plant (Snapshot)

2.1.2 Habitat and Distribution

Ficus glumosa is commonly known as Fig Tree or African Rock Fig [a sacred Fig tree of religious importance in ancient times documented in holy books such as Bible and Qur'an]

(Umar *et al.,* 2013). In Nigeria, it is more distributed in the southern region and is referred to as "Kawuri" in Hausa language (Paul, 2013; Umar *et al.,* 2013), "Obata" in Yoruba, "Obadan" in Edo language (Aigbokhan, 2014), "Akpuru" in Igbo language and "okoklodu" in Idoma language (tribe found in southern part of Benue state in Nigeria). *Ficus glumosa* is indigenous to tropical Africa and sub-tropical Africa, including Nigeria with few species being found in south Asia and in the Mediterranean zone wherethey are usually inhabitant to dried river bed, fringe forest, savannah areas and swamp forest in the coastal regions (Fidele *et al.,* 2014b; Umar *et al*., 2013).

2.1.3 Botanical Description

Ficus glumosa is a small to medium-sized tree, 5-10 m tall, or it may become a large tree reaching 24 m and 2 m in girth (Umar *et al.,* 2013). The branches are widely spreading, more or less horizontal, often supported by stilt roots (Alfred, 2014). The bark is yellow, grey or green-grey, smooth to slightly rough with a few flaking pieces; slash reddish with white streaks; branchlets twiggy, finely hairy to hairy and may be marked with large leaf scars (Orwa *et al.,* 2009). Leaves broadly ovate to oblong, 5-20 x 7-15 cm, green or greyish-green, the young leaves, young branchlets and petioles are frequently with long, tawny, golden to grey silky hairs, giving a shaggy appearance to the young shoots, or hairs may be almost absent (Fidele *et al*., 2014a). Mature leaves usually glabrous above with the lower surface finely hairy, stiffly papery or leathery, and may have scattered long hairs; the ultimate veins forming an extremely fine network, midrib flat above and raised below, with 6-12 pairs of usually distinct, stout lateral veins branched at the ends and looped near the margin, the basal pair more prominent than the rest; apex broadly tapering to rounded; base rounded to shallowly lobed; margin entire; petiole comparatively short, 2.5-9 cm (Tanko *et al.,* 2012). Stipules present, pinkish-brown, conspicuous, velvety, sometimes falling early. Figs small, less than 10-14 mm in diameter, in pairs in the leaf axils and often clustered at the ends of branches among the leaves, globose, paired, glabrous or with fine silky hairs, green turning red, sweet and succulent; stalkless or shortly stalked (Kwazo *et al.*, 2015).

2.1.4 Ethnomedicinal Uses

In Cote d'ivoire, central Africa republic and Zimbabwe, the latex is used to ameliorate pains from sprains, treat diarrhoea and sore eyes, whereas in central Africa, Senegal, east Africa and Tanzania, the stem-bark is used as mouth wash agents to alleviate toothache, to prevent conjunctivitis, treatment of jaundice, dysentery, typhoid fever and stomach disorders (Kwazo *et al.*, 2015). This plant is used in traditional medicine in east Africa, Cameroon and Senegal for the treatment of oedema, hypertension, diabetes, haemorrhoids, rheumatism, skin diseases and stomatitis (Orwa *et al.*, 2009). The leaf is used as vegetable among "Idoma" people (tribe found in southern part of Benue state in Nigeria); it can be eaten after being parboiled with beneseed sauce as a delicacy or used in preparing foods in the forms in which other vegetables are used as well as in traditional medicine for treatment of wounds, inflammation and ailment called "uli" (a disease commonly found among children of under-one that usually results in massive wasting/emaciation).

2.1.5 Some Biological Activities

Some parts (leaf, stem-bark, gum) of *Ficus glumosa* extracts have been previously investigated for some biological activities such as antioxidant activity, anti-diabetic activity, anti-diarrhoeal activity, diuretic activity, haematological activity, hypolidaemic activity and toxicological effects as documented by some researchers.

2.1.5.1 Phytochemicals and Antioxidant Activity

The presence of flavonoids, saponins, tannins and triterpenes in methanol leaves extract were previously reported by Tanko *et al.* (2012). Consequently, Samuel *et al*. (2014a) reported that, the reducing ferric antioxidant power of methanol extract of *Ficus glumosa* stem-bark showed a significant (P<0.05) concentration increase in the total antioxidant power which may be the basis for reducing the elevated thiobarbituric acid reactive substances (TBARS) and increasing the reduced catalase activity in alloxan-induced diabetic rats.

2.1.5.2 Hypoglycaemic and Anti-diabetic Effects

Methanol stem-bark extract and ethanol extract of *Ficus glumosa* leaves investigated against hyperglycaemia induced by alloxan in rats were able to significantly (P<0.05) produced a time-dependent decrease in fasting blood sugar (FBS) as well as significant oral glucose tolerance in normal glycaemic and hyperglycaemic rats which may be of benefit in management of postprandial hyperglycaemia and prevention of diabetic complications (Samuel, *et al.,* 2014a; Umar and Mahaneem, 2015).

2.1.5.3 Anti-diarrhoeal Activity

Previously, study was carried out to investigate the anti-diarrhoeal activity of methanol extract of *Ficus glumosa* leaves using castor oil induced diarrhoea in mice. The extract protected the mice against diarrhoea such that the dose of 20mg/kg body weight produced the highest protection (60%) while40 and 80 mg/kg body weight offered 40 and 20 % protections respectively suggesting the pharmacological activity of methanol leaves extract against diarrhoea (Tanko *et al.,* 2012).The effects of this extract on perfused isolated rabbit ileum were also evaluated where it was found to have produced a dose dependent relaxation of rabbit ileum (Tanko *et al.,* 2012).

2.1.5.4 Diuretic Activity

The aqueous extract of *Ficus glumosa* leaves accelerated the elimination of overloaded fluid and at the maximum diuretic response, urinary osmolarity decreased significantly (P<0.05) while the single dose treatment with aqueous extract of *Ficus glumosa* leaves significantly (P<0.05) increased urine volume after 24 hours of administration when compared with untreated controls (Fidele *et al.,* 2014b). The stability of aldosterone level, the absence of correlation with the plasma levels of sodium, and the increased clearance of free water in the animals receiving the extract showed that, increased diuresis and moderate natriuretic elevation are tubular in origin while the increase in Na^+, K^+, and Cl^-induced by the extract caused alkalinisation of the urine and strong inhibitory effect on carbonic anhydrase and saluretic (Fidele *et al.,* 2014b).

2.1.5.5 Hypolipidaemic and Haematological Effects

Treatment was done orally using 62.5, 125 and 250 mg/kg bw of *Ficus glumosa* stem-bark extract with glibenclamide (2mg/kg bw) as standard reference drug for 21 days to investigate the hypolipidaemic and haematological effects of *Ficus glumosa* by Samuel *et al.*(2014b).All doses of the extract and glibenclamide (2 mg/kg bw) caused dose-dependent and significant (P<0.05) reduction in serum levels of total cholesterol and low density lipoprotein cholesterol (LDL-c) as well as significant (P<0.05) increase in the serum levels of high-density lipoprotein cholesterol (HDL-c) in the treated rats with concomitant increase in red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC) in the extract treated rats when compared to the untreated control group (Samuel *et al.,* 2014b).

2.1.5.6 Toxicological Effects

A single dose (2-12 g/kg bw) of leaves extract of *Ficus glumosa* was administered orally to mice for acute toxicity study, no dose used induced critical behavioural changes or death whereas sub-chronic treatment with daily oral administration of *Ficus glumosa* at the dose of 300, 600, and 1200 mg/kg body weight resulted in a significant (P<0.05) increase in body weight relative to food and water consumption in the last week of treatment but not the relative organ weights (Fidele *et al.,* 2014a). The total cholesterol, triacylglycerol, conjugated bilirubin, and total bilirubin significantly (P<0.05) decreased while the renal function showed a decrease of creatinine, urea, uric acid and Na^+, Cl^- and $Ca^{2+,}$ and inorganic phosphate with normal histology of the liver and the kidney (Fidele *et al.* 2014a).

2.1.5.7 Anti-microbial Activity

Water and methanol extracts of stem-bark of *Ficus glumosa* were screened for their anti-microbial properties against *salmonella* species and consequently, the water extract was found to be more potent based on the phytochemical constituents present and was further subjected to fractionation using gas chromatography where the active fraction that contained 3,3-(4-methyl-1,-3-phenylene)bis(1-heptlurea), isothujol and terbuthyl azine was found to be active against *Salmonella paratyphai* and *Salmonella typhi* (Kwazo *et al.,* 2015).

2.1.5.8 Corrosion Inhibition Effect

The gum of this plant has been reported as a good inhibitor for mild steel corrosion in H_2SO_4 solution using weight loss, thermometric and Scan Electron Microscopictechniques by Ameh(2013) and Alfred (2014). The obtained results showed that inhibition efficiency increases with the rise in temperature and concentration while the mechanism of inhibition effect was due to chemical adsorption and obeyed the Langmuir adsorption model(Ameh, 2013; Alfred, (2014).

2.2 Some Plants with Anti-hepatotoxic Properties in Nigeria

Several species of plants in Nigeria have been evaluated for their anti-hepatotoxic properties with significant ($P<0.05$) protective effects against liver injury arising from chemical substances such as carbon tetrachloride, acetaminophen, cadmium, ethanol, alloxan and dimethyl nitrosamine. Findings from some of these evaluations on some plant species are summarised according to their families as follows:

Amaranthaceae is a family of flowering plants that includes trees, shrubs, herbs and stem such as *Alternantheradendata*(Aigbokhan, 2014). Comparative analysis of hepatoprotective effects of ethanol leaves extract of *Gongronemalatifolia* and *Alternantheradendata* showed that,*A. dendata* leaves extract significantly ($P<0.05$) reduces elevated liver function parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, albumin) and tends to increase the total protein concentration in the serum of non-intoxicated rats, an effect that was attributed to the presence of some important phytochemicals such as flavonoid, tannins and alkaloid in the extract that are perceived to exhibit antioxidant activity (Sylvester, 2015).

Amaryllidaceae are members of herbaceous plants usually perennial and bulbous flowering plants including species like *Allium cepa* and *Allium sativa*. These two species (*Allium cepa* and *Allium sativa*) have been examined using their methanol bulb extracts against hepatotoxicity induced by paracetamol in experimental rats. The administration of bulb extracts of these species to rats significantly ($P<0.05$) reversed liver damage induced by paracetamol in experimental rats as evident in significant reduction of liver marker enzymes activities in the serum of the extract treated animals as compared to the untreated intoxicated animals indicating the future clinical hepatoprotective effects of these species (Jevas *et al.,* 2014; Ozougwu and Eyo, 2014).

Annonaceae family including *Annona senegalensis, Enantia chlorantha* and *Uvaria afzelii* were previously investigated using the root-bark, stem-bark and root of these species respectively for anti-hepatotoxic potentials via paracetamol and CCl_4 induced liver damage experimental model in rats. Methanol-methylene root-bark extract of *Annona senegalensis* was able to significantly ($P<0.05$) reduced liver function indices in liver damaged rats (Theophine *et al.,* 2012) as it was in the case of stem-bark extract of *Enathia chlorantha* that equally mitigated liver injury caused by acetaminophen as evident in the reduction of serum activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and decrease in the concentration of bilirubin as well as significant increase in total serum protein and repair of histological features of the liver in the experimental rats (Olamide and Mathew, 2013). Similarly, in CCl_4 intoxicated rats, the methanol root extract of *Uvaria afzelli* did significantly (P<0.05) restore normalcy of liver function assessment parameters and the structural architecture in the extract treated experimental animals as compared to the non-treated intoxicated animals (Ofeimun *et al.,* 2013)

Apocyanaceae (Gongronema latifolia, Taccazzea barteri): Ezeonwu and Dahiru (2013) demonstrated that administration of acetaminophen increases the serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, creatinine, urea with reduction in serum total protein in rats which were significantly (P<0.05) reversed on treating with aqueous leaves extract of *Gongronema latifolium.* Similarly, in a non-intoxicated comparative study of *Alternanthera dentata* and *Gongronema latifolia* where ethanol leaves extract of *Gongronema latifolium* was administered to experimental rats; it was found out that different doses of the leaves extract of *G. latifolium* significantly (P<0.05) reduced the serum activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, albumin and increase the total serum protein (Sylvester, 2015) likewise, in a CCl_4-induced hepatotoxic rats, aqueous leaves extract of *Taccazzea barteri* equally exhibited the same effect with significant (P<0.05) reversal of the injury caused to the histology of the liver (Obioha *et al.,* 2013).

Araceae (Colocasia esculentus): Aqueous leaves extract of*Colocasia esculentus* as examined against thioacetamide-induced toxicity showed protective effect by normalising the liver enzymes markers. The result obtained from the activity of *C. esculentus* aqueous leaves extract was comparable to that of the hepatoprotective standard drug, silymarin that significantly (P<0.05) lowered serum activity of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and increased the serum concentration of albumin as compared to the animals challenged with thioacetamide without any form of treatment (Azubike *et al.,* 2015).

Asteraceae (*Vernonia amygdalina,Tridax procumbens, Emilia sonchofolia*): Dimethylnitrosamine produced liver damage in rats as manifested by significant (P<0.05) rise in the levels of alanine aminotransferase, total cholesterol with concomitant decrease in total protein, globulin, albumin, white blood cells, red blood cells, platelets, haemoglobin and packed cell volume in rats however, administration of *Vernonia amygdalina* ethanol leaves extract significantly (P<0.05) reversed these parameters towards normal (Usunobun, 2014). Similarly, aqueous leaves extract of *V. amygdalina* was able to significantly (P<0.05) reduced liver marker enzymes,malondialdehyde (MDA) concentration and increase the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH) and as well as increasing the concentrations of red blood cells and haemoglobin with decrease in white blood cells in rats induced with acetaminophen (Momoh, *et al.,* 2015). In the same way, aqueous leaves extract of *Tridax procumbens* protects the liver against carbon tetrachloride induced hepatotoxicity by exhibiting similar biological activity as in the case of *V. amygdalina* against biochemical parameters in rats (Jude, 2012), similarly,*Emilia sonchofolia*leaves extract was able to exhibit same liver protection effect in mice infested with *Plamodium berghei berghei* (Innocent*et al.,* 2014).

Bignoniaceae (*Newbouldia laevis):* The decrease in endogenous antioxidant enzymes (GSH, CAT), serum total protein, vitamin C, E, albumin, and increase in lipid peroxidation (MDA), aspartate aminotransferase, alanine aminotransferase, bilirubin (both total and direct bilirubin), cholesterol levels observed in the serum of rats induced with carbon tetrachloride without treatment were noticed to be significantly (P<0.05) reduced upon administration of leaves and stem-bark extract of *Newbouldia laevis* (Hassan *et al.,* 2010 and 2015).

Cesalpiniaceae (*Cassia italic):* Pre-treatment of rats with *Cassia italica* ethanol leaves extract by Nadro and Onoagbe (2014) was found to significantly (P<0.05) lowered liver function enzymes marker (AST, ALP, ALT), lipid peroxidation (MDA), cholesterol levels and increase albumin level in the experimental rats challenged with CCl_4 as compared to the control group that were induced with CCl_4without pre-treatment of ethanol leaves extract of *C. italica*.

Chrysobalanaceae (Parinari curatellifolia): Evaluation of antioxidant and hepatoprotective effects of *Parinari curatellifolia* methanol root extract by Atawodi *et al.,* 2013 showed a useful liver protective activity against carbon tetrachloride-induced hepatotoxicity in rats. Methanol root extract of *P. curatelliafolia* was able to significantly (P<0.05) reduced the elevated levels of MDA, AST, ALP and bilirubin as well as increase the superoxide dismutase (SOD) and catalase levels in the extract treated animals as compared to the untreated animals (Atawodi *et al.*, 2013).

Connaraceae (*Cnestis ferruginea):*It was evident from the study conducted by Akharaiyi *et al.*(2012), that liver function marker enzymes (AST, ALP, ALT) in mice treated with ethanol extract of *Cnestis ferruginea*leaves after paracetamol induction were significantly(P<0.05)lowered as compared to those mice intoxicated with paracetamol but were neither treated with the ethanol extract of *Cnestis ferruginea*leaves nor standard drug.

Cyperaceae (Cyperus esculentus): Similarly, seed extract of *Cyperus esculentus*administered to rats for 21 days prior to the induction of hepatic injury by carbon tetrachloride was able to significantly (P<0.05) prevent the elevation of liver marker enzymeswhen compared to those rats that were not pre-treated with the extract (Oyedepo and Odoje, 2014).

Euphorbiaceae (*Alchornea cordifolia, Jatropha tanjorensis, Cnidoscolus aconitifolius):* The hepactoprptective property of *Acalypha wilkesiana* was evident in the reduction of high levels of AST, ALP, ALT, bilirubin and increase in total protein in aqueous leaves extract treated animals as compared to the levels of these parameters in the untreated animals (Nwaoguikpe *et al.,* 2015). Hepatocyte necrosis, cellular infiltration and inflammation in the liver of rats intoxicated with acetaminophen were returned to their normal lobular architecture following treatment with ethanol leaves extract of *Alchornea cordifolia* (Jacob et al., 2014). Also, the leaves extract of *A. cordifolia* were equally proven to be potent in lowering elevated levels of liver marker enzymes in both paracetamol and carbon tetrachloride-induced hepatotoxicity in rats (Patience *et al.*, 2012; Arhoghro *et al.,* 2015). Similarly, treatment of carbon tetrachloride induced hepatotoxicity in experimental rats with methanol leaves extract of *Jatropha tanjorensis* significantly (P<0.05) reduced the elevated serum levels of AST, ALP, ALT, total cholesterol, triacylglycerol, low density lipoprotein cholesterol and increases high density lipoprotein cholesterol comparable to those of silymain (Madubuike *et al.*, 2015). Saba *et al.* (2010) equally showed the hepatoprptective potentials of leaves extract of *Cnidoscolus aconitifolius* in rats where it was found to be potent against hepatotoxicity induced by carbon tetrachloride by reversing liver enzymes to a near normal.

Fabaceae (Millettia oboesis, Tetrapleura tetraptera, Zapoteca portoricensis): Evaluation of the hepatoprotective activity of *Millettia oboesis* on CCl_4-induced hepatotoxicity in rats confirmed protective ability of the extract by the lowering of the increased levels of AST, ALP, ALT and bilirubin comparable to the standard drug, LIV-52 (Ugwueze *et al.,* 2013). There was also an improvement in the liver function parameters when rats that were induced with alloxan were treated with leaves extract of *Tetrapleura tetraptera* as it was evident in the significant (P<0.05) reduction of serum levels of AST, ALP, bilirubin, urea, creatinine, malondialdehyde with increase in the levels of catalase, superoxide dismutase, packed cell volume and high density lipoprotein cholesterol as compared to the reverse status of these parameters in the untreated animals (Atawodi *et al.,* 2014). Similarly, *Zapoteca portoricensis* leaves extract was able to reverse both the elevated serum activities of liver marker enzymes and the reduced antioxidant enzymes status as seen in the untreated rats to a near normal in the treated rats (Kingsley *et al.*, 2014).

Guttiferae (Mammea Africana): Administration of ethanol stem-bark extract of *Mammea africana* caused a significant (P<0.05) dose dependent reduction of high levels of liver enzymes (ALP, AST, ALT), total cholesterol, total and direct bilirubin, as well as elevation of serum levels of total protein, albumin, antioxidant enzymes (SOD, CAT, GPx, GSH) with reduction in the injury to the pathological features of the liver as compared to their levels observed in the untreated rats induced with paracetamol (Jude*et al*., 2016).

Lamiaceae (Hyptis suaveolens, Ocimum americanum,, Ocimum gratissimum): Paracetamol was found to significantly (P<0.05) increase liver enzymes (ALP, AST, ALT), total bilirubin and decrease total protein and albumin in experimental animals, however, upon treatment with aqueous leaves extracts of *Hyptis suaveolens* and *Ocimum americanum,* the status of these indices in the animals were found to have reversed towards normal (Babalola *et al*., 2011; Aluko *et al.*, 2013). Similarly, biochemical analysis showed significant (P<0.05) increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), creatinine, urea, cholesterol and triglyceride with reduction in total protein levels of rats administered with acetaminophen without extract pre-treatment, meanwhile, there was a significant (P<0.05) dose dependent reversal of these changes in rats pre-treated with the bi-herbal aqueous leaves extracts of *Ocimum gratissimum* and *Gongronema latifolium* (Ezeonwu and Dahiru, 2013).

Legumunoseceae (*Cassia occidentalis*, *Pterocarpussantalinoides*, *Xylopia aethiopia):* Microscopy revealed normal histological hepatocytes in the control animals that were not induced with paracetamol while those of test control that were induced and left untreated revealed severe vascular congestion, periportal infiltrates of chronic inflammatory cells and periportal oedema (Uzzi and Grillo, 2013). However, hepatic sections from groups induced but treated with ethanol leaves extract of *Cassia occidentalis* presented a dose dependent healing actions compared to the features observed for untreated hepatotoxic group (Uzzi and

Grillo, 2013). Similarly, there were significant (P<0.05) reductions in aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP) activities in rats administered with ethanol leaves extract of *Pterocarpussantalinoides* in a dose dependent manner as comparedto the significant (P<0.05) elevated activities of AST, ALT and ALP recorded in the group that received CCl_4 without treatment (Aja *et al.*, 2016). Iwuanyanwu *et al*. (2010) reported that malondialdehyde concentration was significantly (P<0.05) reduced in addition to the reversal of liver function indices in rats pre-treated with fruit extract of *Xylopia aethiopia* followed by CCl_4 intoxication in rats.

Lythaceae (*Lawsonia inermis): Lawsonia inermis* extract significantly (P<0.05) decreased the serum levels of AST and ALT, even though not in a dose dependant manner, suggesting that aqueous leaves extract of *Lawsonia inermis* has hepatoprotective effects at appropriate dosage against hepatotoxicity induced by carbon tetrachloride (Sanni*et al.,* 2010).

Meliaceae (Swietenia mahogany , Azadirachta indica): Aqueous leaves extract of *Swietenia mahogany*extract significantly (P<0.05) reduced the serum activities of ALT, AST, AP, serum levels of bilirubin and creatinine with shortening of the duration of pentobarbital induced hypnosis in treated rats as compared to the control group that received alcohol without treatment (Samuel *et al.*, 2011). Oral administration of acetaminophen to rats caused marked liver damage as noted by the significant (P<0.05) increase in activities of plasma AST, ALT, ALP and GGT as well as the level of cholesterol, triglyceride and reduction in plasma total protein with significant (P<0.05) increase in liver MDA content, decrease in liver GSH, SOD and CAT activities (Momoh *et al.*, 2015). However, treatment with silymarin, Vitamin C, *Vernonia amygdalina* and *Azadirachta indica* extracts showed effective hepatoprotective effect as seen in the decreased plasma levels of liver biomarker enzymes and significant (P<0.05) reversal of oxidative stress parameters with reduced incidence of paracetamol-induced liver lesions as revealed by the histopathological analysis (Momoh *et al.,* 2015).

Moraceae (*Ficusexasperate):*Biochemical evaluation of rats challenged with paracetamol but treated with *Ficusexasperata* ethanol leaves extract showed a significant (P<0.05) decrease in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities and total bilirubin (TB) concentration in a non-dose dependent manner when compare with the untreated group (Odutuga *et al.,* 2014). Similarly, the stem-bark extract of *Ficusexasperate* protects the liver against paracetamol induced toxicity in wistar rats (Enogieru *et al.*, 2015).

Moringaceae (*Moringa oleifera):*Substantially elevated serum activities of hepatic marker enzymes (ALT and AST) in CCl_4-intoxicated rats were significantly (P<0.05) lowered towards normal values by the administration of *Moringa oleifera* seed oil which indicated its remarkable hepatoprotective potential underlining its ability to reverse CCl_4-induced liver injury in the experimental animals as it was also evident in the histopathological appearance (Olatosin *et al.,* 2013).

Onagraceae (*Jussiaea nervosa):* Liver function parameters (ALT, AST, ALP and bilirubin) were significantly (P<0.05) elevated withreduction in the total protein and albumin in rats exposed to cadmium toxicity without treatment (Ama *et al.,* 2013). *Jussiaea nervosa* extract however, lowered the values of liver function parameters while the serum albumin and total protein were significantly (P<0.05) increased when the animals were treated with the extract (Ama *et al.*, 2013).

Phyllanthaceae (*Phyllanthus amarus):* Adaeze *et al*.(2015) reported a significant (P<0.05) increase in the levels of Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), with a reduction in total protein (TP) levels of rats as a result of acetaminophen administration. Meanwhile, the status was significantly (P<0.05) reversed comparable to silymarin effect by*Phyllanthus amarus* aqueous leavesextract treatment (Adaeze *et al*., 2015).

*Piperaceae (Piper guineense):*Chronic administration of ethanol resulted in enhanced lipid peroxidation with depletion in the level of GSH as well as reduction in the activities of SOD and Glutathione-S-transferase with elevation of triacyglyceride level, ALT and AST activities in rats that were only given ethanol however, the circumstances were significantly(P<0.05) attenuated by the co-administration of*Piper guineense* seed extract by oral gavage in other group of rats (Sarah *et al.*, 2012).

*Rubiaceae (Heinsia crinite, Morinda lucida, Nauclea latifolia):*Evaluation of methanol leaves extract of *Heinsia crinita*for hypoglycemic, hepatoprotective and nephroprotective effects against alloxan-induced diabetes complications in rats by Ebong *et al.*(2014) showed significant (P<0.05) decrease in ALT, AST and ALP activities as well as elevation of K^+ and Cl^- but lowering of Na^+ions and urea value in extract treated groups when compared with non-treated group which gave high values of these enzymes as well as low values of K^+ and Cl^- but high values of Na+ and urea. Similarly, findings by Adejo *et al.* (2014) showed that, the anti-peroxidative and protective properties of *Morinda lucida*are comparable to Vitamins C and E, while the ameliorative properties are comparable to Silymarin effect against liver injury induced by carbon tetrachloride in rats. Furthermore, levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated whereas the levels of total protein, albumin, activities of catalase (CAT), glutathione Peroxidase (GPx) and superoxide dismutase (SOD)were all significantly (P<0.05) decreased in acetaminophen treated rats, however,*Nauclea latifolia* ethanol leaves extract significantly (P<0.05) decreased the elevated levels of the transaminases and restored the normalcy of total protein (TP), albumin and antioxidant status (Effiong *et al.*, 2013).

Tiliaceae (*Corchorus olitorius*): Serum enzymes assay for liver function assessment in carbon tetrachloride hepatotoxic rats revealed marked reduction in the elevated activity of the hepatic enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)] levels in experimental rats treated with ethanol leaves extract of *Corchorus olitorius*comparableto the normal rats (Ujah *et al.*, 2014).

2.3 Silymarin

Silybum marianum is a member of the *aster* family (*Asteraceae*) with leaves characterised by milky veins from which it derives the name 'milk thistle' (Lidia and Wojciech, 2007). Silymarin is a polyphenolic flavonoid extracted from the seeds of *Silybum marianum* that has been used traditionally for centuries as herbal medicine for the treatment of liver cirrhosis, alcoholic and viral hepatitis, liver poisoning and gallbladder disorders (Luminita *et al.*, 2011). The seeds of *silybum marianum* contain approximately 70-80% of sylimarin flavonolignans made up of four isomers [silybin, isosilybin, silydiannin and silychristin] (Figure 2.2) and 20-30% of polymeric and oxidized polyphenolic compounds (Nitin *et al.*, 2007).Silymarin is slowly absorbed from the gastrointestinal tract (23-47% absorbed) following oral administration such that the peak plasma concentrations are reached after 2 hours while the elimination half-life is 6-8 hours, 3-8 % of it is eliminated through urine and 20-40 % is recovered from the bile as glucouronide and sulphate conjugates (Ghosh, *et al.*, 2010; Nitin *et al.*, 2007).

2.3.1 Mechanisms of Action of Silymarin

Mechanisms of action of silymarin can be summarised to include the following actions: ability to regulate membrane permeability and increase its stability, inhibition of transformation of stellate cells in the liver into myofibroblasts, activity against lipid peroxidation by free radical scavenging and ability to increase the cellular concentration of reduced glutathione, stimulation of ribosomal RNA polymerases, enhance glucoronidation, immunomodulatory effects and inhibition of leukotriene production (Valenzuela *et al*., 1989; Muriel *et al*., 1990; Muriel *et al*., 1992; Hikino *et al*., 2000; Ghosh, *et al*., 2010).

Antioxidant activity- silyimarin acts as antioxidant by scavenging free radicals, raising the cellular content of glutathione to intensify its biological functions and stimulating superoxide dismutase activity via adjustment of nuclear expression that in turn lead to inhibition of lipid peroxidation and increase membrane stability in exposure to xenobiotics (Gholamreza, *et al.,* 2011).

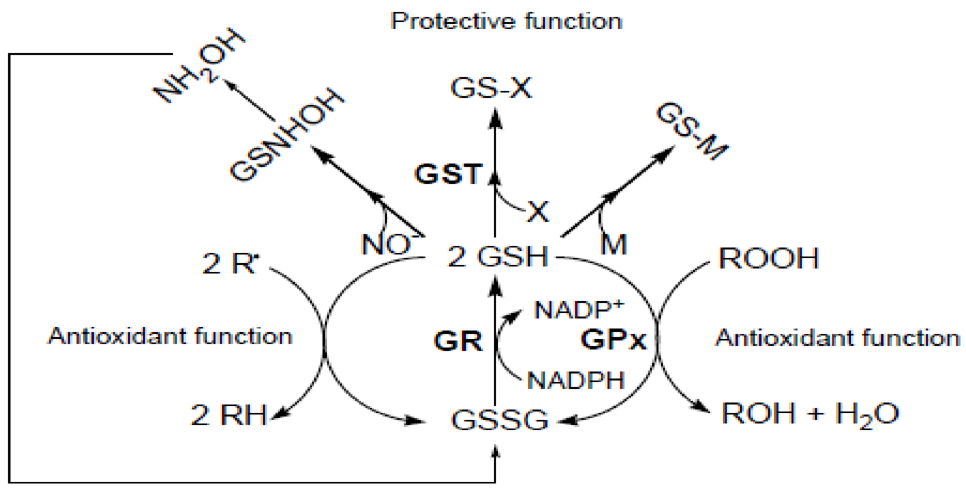


Figure 2.1: Biological Function of Glutathione (Ivana *et al.*, 2010)

Stimulation of protein synthesis- silymarin facilitates the activity of RNA polymerase I enzymes and the transcription of ribosomal RNA in the nucleus which increase ribosomal formation and consequently improvesbiosynthesis of both structural and functional proteins as well as cytoplasmic apparatus, thus initiating damaged tissues repair leading to restoration of normal tissue functions (Lidia and Wojciech, 2007; Gholamreza, *et al.*, 2011).).

Anti-fibrotic effect- fibrogenesis is basically enhanced by the conversion of hepatic stellate cells into myofibroblast which reduces the expression of profibrogenic procollagens alpha I and the tissue inhibitor of metalloproteinase I probably by down regulation of profibrogenic cytokine, TGF-β (Ghosh, *et al.,* 2010). Silymarin limits production of mediators such as reactive oxygen species and leukotriene in kuffer cells that promote stellate cells activation and proliferation by intercepting the reactive oxygen species and inhibiting 5-lipoxygensae, an enzyme that catalysis leukotriene formation from arachidonic acid (Luminita *et al.,* 2011).

Anti-toxic effect- silymarin exerts a regulatory action on cellular and mitochondrial membrane permeability as well as stability that prevents absorption of xenobiotics into hepatocyte by occupying the binding site and inhibiting many transport proteins at the membrane (Lidia and Wojciech, 2007).

Anti-cancer activity- the mechanism of anti-proliferation activity of silymarin is related to radical scavenging effects as well as the specific receptor interaction and modulation of a variety of cell signalling pathways (Gholamreza, *et al.,* 2011) such as NF-kappa B (Nuclear Factor kappa B-cells), EGFR-MAPK (Epidermal Growth Factor Receptor-Mitogen Activated Protein Kinases) signalling and IGF-receptor signalling (Insulin-like Growth Receptor)pathways (Gazak, *et al.,* 2007).Down-regulation of EGFR signalling by silymarin and silibinin occurs via various mechanisms such as the inhibition of growth factors expression and secretion, preventing growth factor binding and activation of EGFR and destruction of mitogenic procedures causing anti-cancer effectiveness in tumour cells (Rasmasamy and Agarwal, 2008)). Similarly, silymarin and silybin exhibit anti-angiogenic activity by mechanism of decreasing of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) secretion (Kaur and Agarwal, 2007).

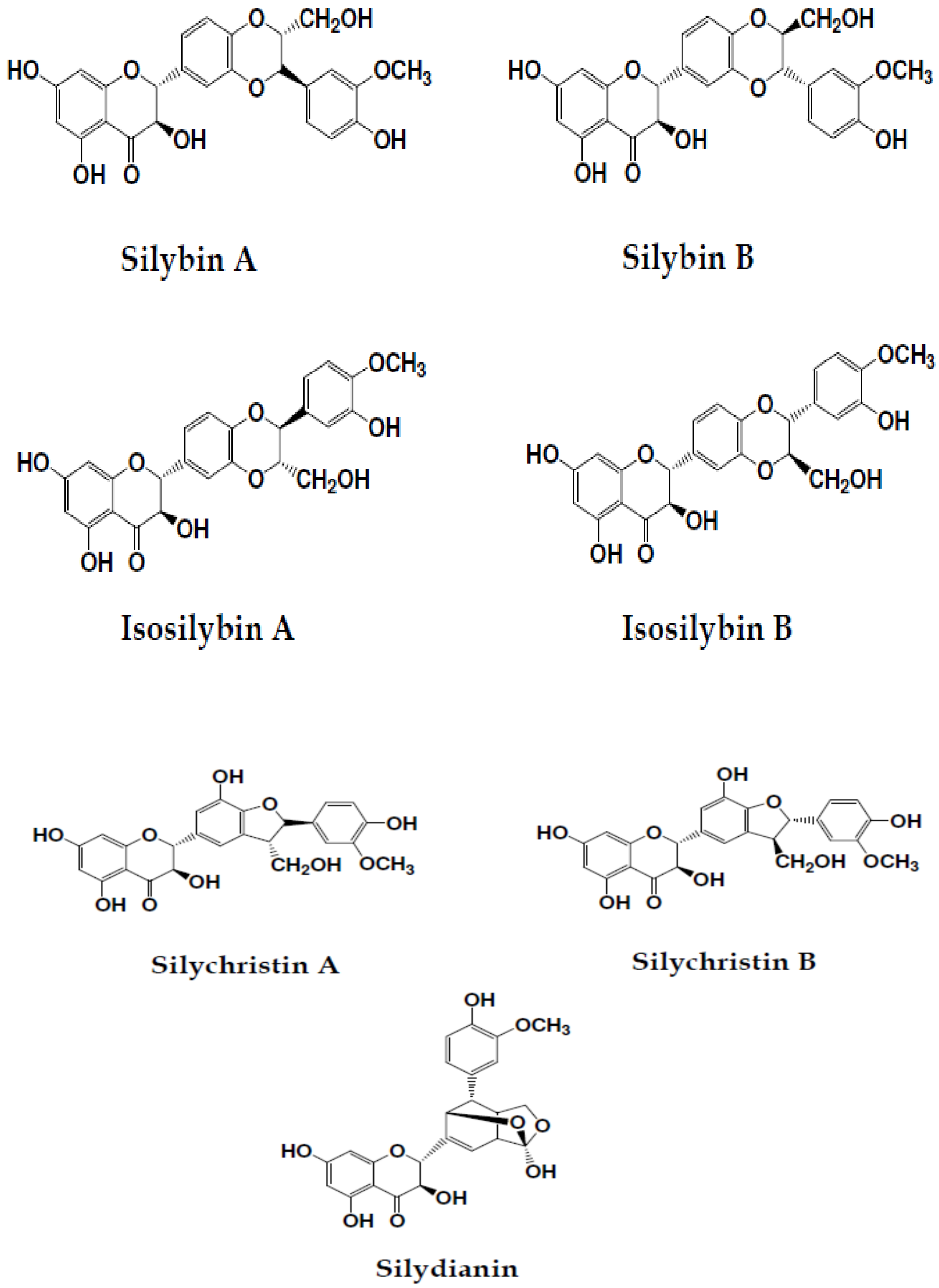


Figure 2.2: Chemical Structures of Silymarin Components (Ghosh *et al*., 2010)

2.4 Carbon Tetrachloride Metabolic Fate

Carbon tetrachloride in the presence of reduced form of cytochrome may undergo one electron reduction to generate free radical that may be transformed by different pathway to several reactive intermediates such as peroxides, carbenes and radicals whose participation in toxic effect in the biological system varies greatly (Macherey and Dansette, 2008).

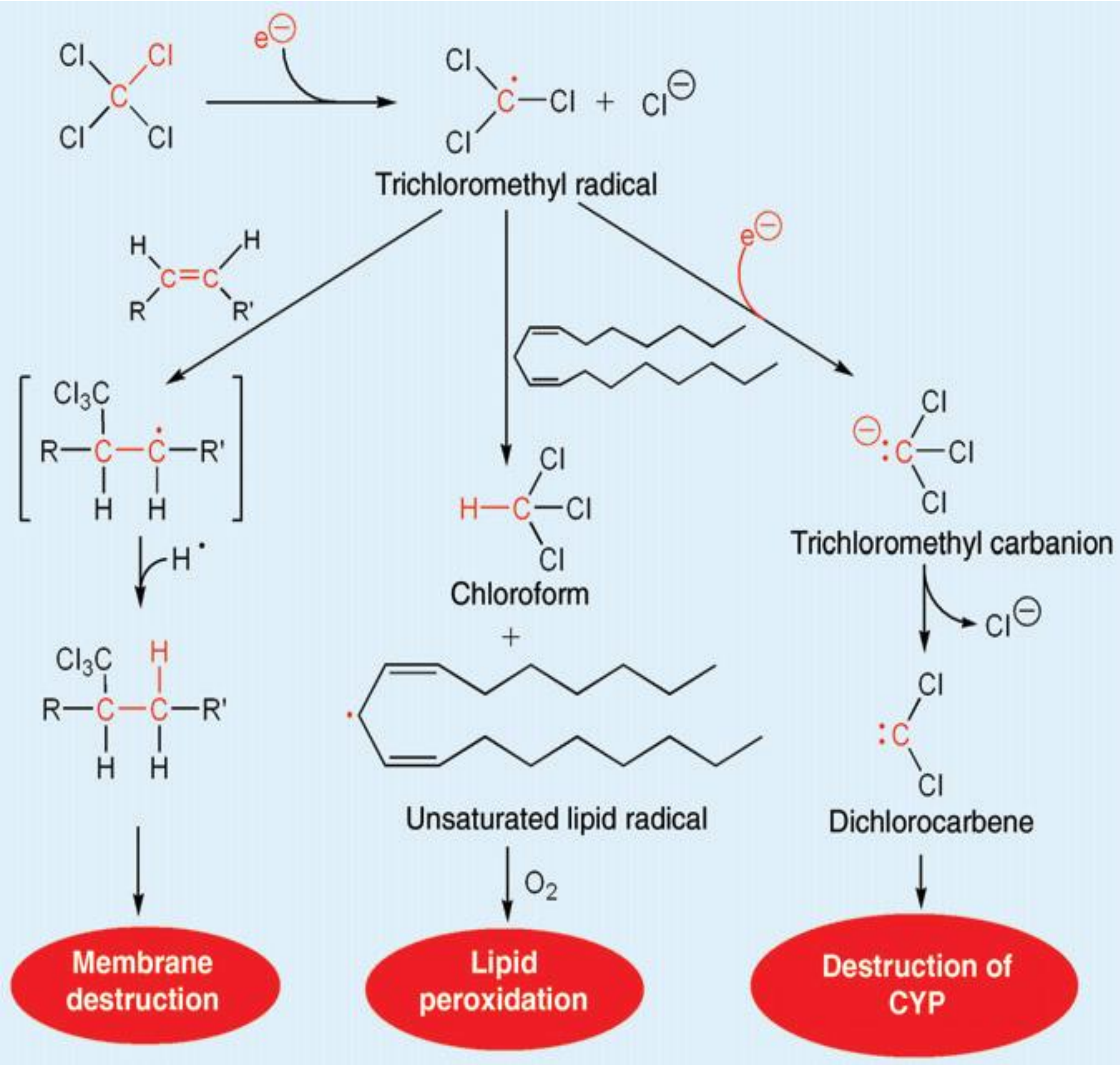


Figure 2.3: Reduction of Carbon Tetrachloride (Macherey and Dansette, 2008)

2.4.1 Theories of Mechanisms of Action of Carbon Tetrachloride

Many studies including Recknagel, *et al.* (1989), Weber, *et al*. (2003), Manibusa, *et al.* (2007) reported that CCl_4 toxicity is a multifactorial process involving several mechanisms of action which include some of the following summarised actions:

Oxidative stress and lipid peroxidation- Carbon tetrachloride is activated by cytochrome P_{450} monooxygenase system to form trichloromethyl (CCl_3°) free radical that can further react with oxygen species to produce trichloromethyl peroxy radical (CCl_3OO°) (Laetitia *et al.,* 2012). Both radicals are highly reactive that may covalently bind macromolecules to form nucleic acid, protein and lipid adducts (Weber *et al.*, 2003; Manibusa *et al*., 2007). As regards to lipid damage, CCl_3OO° radical initiates lipid peroxidation by pulling out hydrogen atom in the vicinity of polyunsaturated fatty acid double bond found in the cell (Laetitia *et al*., 2012). After propagation of peroxidation process, lipids are finally degraded in small molecules such as malondialdehyde (MDA) or in 4-hydroxynonenal (HNE), which are highly reactive aldehydes that can form protein and DNA adducts (Levy, 1984; Kadiiska *et al.,* 2005).

Obstruction of calcium homeostasis- carbon tetrachloride and its radicals induce toxicity in the liver cells by inhibiting Ca^{2+}/Mg^{2+}-ATPase in the endoplasmic reticulum which leads to a mass decrease in the concentration of calcium in microsomes with increase in concentration of calcium in the cytosol thereby disrupting the homeostasis of calcium in the liver (Recknagel *et al.,* 1989; Manibusa *et al.,* 2007). In addition, there is loss of cellular calcium, sequestration and disruption with subsequent cellular damage due to alteration in the permeability of mitochondrial, endoplasmic reticulum and plasma membranes caused by lipid peroxidation induced by CCl_4 (Weber *et al*., 2003).

Activation of cell proliferation- carbon tetrachloride has been reported to activate tumour necrosis factor (TNFα), nitric oxide (NO) and transforming growth factors (TGFα and TGFβ) in the cell thereby promoting cellular destruction in the form of apoptosis and proliferation in the form fibrosis (Saba *et al.,* 2010).

Protein synthesis inhibition- carbon tetrachloride inhibits mRNA transport which in turn decreases the NTPase activity of the nuclear envelope and consequently inhibits protein biosynthesis in the cell (Xia-Wen *et al.,* 2010).

Alteration of lipid metabolism- carbon tetrachloride causes fatty degeneration of the liver cells via lipid accumulation which begins at the early stage of CCl_4 intoxication due to failure of the liver to transport triacyglyceride low density lipoproteins secretion as a result of structural disorganisation of the endoplasmic reticulum which causes loss of microsomal enzymes functions such as cytochrome P450 monooxygenase system and glucose-6-phosphatase (Recknagel *et al.*, 1989).

Genotoxicity- carbon tetrachloride interferes with chromosome segregation leading to aneuploidy, increases intra-chromosomal recombination, causes double stranded DNA breakage and formation of DNA/RNA adducts (Kadiiska *et al.*, 2005; Mariam *et al.*, 2015).

2.5 Liver and its Functions

The liver is the largest organ in the human body, weighing between 1200 – 1500g (Thirupathi *et al.*, 2011). It is the key organ in homeostasis within the body that regulates several vital functions including synthesis, storage, metabolism of macromolecules as well as detoxification and excretion of endogenous and exogenous (xenobiotics e.g drugs) harmful materials (Thirupathi *et tal.*, 2011; Vasudevan *et al.*, 2011). Liver disorders are associated with distortion of these important metabolic processes which may subsequently lead to accumulation or insufficient metabolites (Ward *et al.,* 2005) and such interference with metabolites may mark the pathogenesis of several disorders such as hyperlipidaemia, jaundice, oedema and inflammation. The interactions of liver enzyme system cytochrome P450 (CYP450) with foreign harmful compounds most often result to the formation of metabolites that may be more reactive and toxic to the cells than the parent compounds (Heard, 2008). For instance, paracetamol usually undergo oxidation reaction by CYP450 enzymes in the liver to generate N-acetyl-p-benzo-quinoneimine (NAPQI) which is the actual compound responsible for the toxicity arising from the overdose of paracetamol (Mitchell *et al.*, 1973; Heard, 2008). Today, liver damage is one of the common ailments in the world resulting in several mild and serious metabolic disorders that could lead to death (Raheim *et al.,* 2013). Being a versatile organ concerned with regulation of internal chemical environment, damage inflicted on it by hepatotoxic agent may pose a grave consequence (Sreedevi *et al.,* 2009).

2.6 Kidney and its Functions

The kidneys are bean-shaped organs located at the rear of the abdominal cavity in the retroperitoneal space that are essential to the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance and regulation of blood pressure via maintaining the salt and water balance (Biff 2015; Vasudevan *et al.,* 2011). They serve the body as a natural filter of the blood, and remove water-soluble wastes such as urea and ammonium which are diverted to the bladder as urine (David *et al.,* 2015). They are also responsible for the reabsorption of water, glucose, and amino acids back into circulation as well as production of hormones (including calcitriol and erythropoietin) and important enzyme (renin) which acts in negative feedback (Le, 2013). Renal failure interferes with these essential functions resulting in inefficient removal of waste products and imbalance in osmotic pressure. This can occur quickly (acute renal failure), or gradually (acute kidney injury) often as the result of ischaemia, toxins or mechanical trauma (David *et al.,* 2012). The kidney is highly energetic and therefore relies heavily on aerobic metabolism for the production of ATP by oxidative phosphorylation.

Kidneys play a very key role in human osmoregulation by regulating the amount of water and ions reabsorbed from glomerular filtrate in kidney tubules, which is controlled by hormones such as antidiuretic hormone (ADH), aldosterone, and angiotensin II (Erik *et al,* 2014). A decrease in water potential of blood is detected by osmoreceptors in hypothalamus, which stimulates ADH release from pituitary gland to increase the permeability of the wall of the collecting ducts in the kidneys through activation of aquaporin membrane proteins(David *et al.,* 2015). Therefore, a large proportion of water is reabsorbed from fluid to prevent a fair proportion of water from being excreted. Whereas, increase in water potential of the blood do not generate ADH and hence results in fluid excretion from the body through urine. By so doing, the two opposing actions will then maintain a balanced osmotic pressure in the blood.

2.7 Oxidative Stress

Oxidative stress is a phenomenon associated with pathogenesis of several diseases including atherosclerosis, ulcerative colitis, neurodegenerative diseases such as Alzheimer's and Parkinson's disease, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or aging processes (Maritim *et al.,* 2003; Polidori *et al.,* 2007; Sandra *et al.,* 2009; Subash *et al.*, 2010; Hamouda *et al.,* 2011; Rana *et al.*, 2014). Oxidative stress is defined as an imbalance between production of free radicals or reactive metabolites (oxidants) and their elimination by protective mechanisms, referred to as antioxidative systems (Rana *et al.,* 2014; Patil *et al.,* 2007). This imbalance leads to damage of important biomolecules and organs with potential impact on the whole organism. Oxidative and antioxidative processes are associated with electron transfer influencing the redox state of cells and the organism (Salwa and Abass, 2011).

2.7.1 Free Radicals

Free radicals are species (atoms, molecules, ions) that have one or more unpaired electrons in their outer orbital that make them very unstable and quite reactive with other molecules by pairing up their electron(s) to generate a more stable compound (Salwa *et al.,* 2011). Reactive oxygen species (ROS) or free radicals, formed during physiological and pathological conditions in the body are extremely reactive and react with proteins, lipids, carbohydrates and nucleic acids (Carmen *et al.,* 2012; Laetitia *et al.,* 2012). Free radicals are derived from oxygen, nitrogen and sulphur to form reactive oxygen species, reactive nitrogen species and reactive sulphur species respectively. The nitrogen derived free radicals include nitric oxide (NO), peroxy nitrite anion (ONOO), nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3); the thiol derived free radicals include sulphite (SO_3^{2-}), disulfide S oxide (DSSO), sulfenic acid (RSOH) and sulfenyl (RS.) radicals (Lu *et al*., 2010). When the body is overloaded with free radicals and cannot be gradually destroyed, their accumulation in the body generates a phenomenon called oxidative stress (Carmen *et al.,* 2012).

2.7.2 Mechanisms of Oxidative Damage by Free Radicals

- By homolytic cleavage of covalent bond of normal molecule, with each fragment retaining one of paired electrons.
- By the loss of single electron from normal molecule.
- By addition of single electron to normal molecule **(**Recknagel *et al.*, 1989; Weber *et al.,* 2003; Manibusa *et al.*, 2007)

2.7.3 Oxidative Damage to Lipids by Free Radicals

All of the most important classes of biomolecules may be attacked by free radicals but lipids are probably the most sensitive. Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs) which are readily attacked by oxidising radicals (Vasudevan *et al.,* 2011). The oxidative destruction of PUFAs is known as lipid peroxidation which involves pulling out of hydrogen atom(s) from the vicinity of polyunsaturated fatty acid double bond which is particularly damaging because it proceeds as a self-perpetuating chain-reaction (Laetitia *et al.,* 2012).

2.7.4 Oxidative Damage to Proteins by Free Radicals

Oxidative attack on proteins results in site-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross linked reaction products, altered electrical charges and increased susceptibility to proteolysis (Lobo *et al.,* 2010; Carmen *et al.,* 2012).

2.7.5 Oxidative Damage to DNA by Free Radicals

Activated oxygen species and agents that generate oxygen free radicals, such as ionizing radiations, induce numerous lesions in DNA that causes deletion, mutations and other lethal genetic effects (Craft *et al.,* 2012) similarly, characterization of this damage to DNA has indicated that both sugar and base moieties are susceptible to oxidation, causing base degradation, single strand breakage and cross links to proteins (Lu *et al.,* 2010).

2.8 Antioxidant System

Antioxidants are exogenous or endogenous compounds which either prevent or delay the generation of toxic oxidants or intercept those that are already generated to inactivate them, thereby blocking the chain of propagation reaction by these oxidants (Halliwell, 2010). They can also initiate repair processes (e.g damaged DNA repaired by sulphaoxide reductase) which remove damaged macromolecules to prevent their accumulation that may further hinders cellular process and viability (Subash *et al.,* 2010). They have ability to protect human body cells from the damages caused by unstable free radicals (highly reactive chemicals that play part in generating oxidative stress in biological system) by stabilizing them via electron donation (Lu *et al.,* 2010).

2.8.1 Classification of Human Antioxidants According to the Source

According to the source, antioxidants could be classified into: (a) endogenous (b) exogenous

2.8.1.1 Endogenous Antioxidants

The endogenous group are found within the human biological system and they include metallo-enzymes superoxide dismutase (zinc, manganese, and copper), glutathione peroxidase (selenium) and catalase, and proteins like albumin, transferrin, ceruloplasmin, metallothionein and haptoglobin(Halliwell, 2010).

2.8.1.2 Exogenous Antioxidants

The most essential form of exogenous antioxidants are dietary phytochemicals(such as polyphenols, quinones, flavonoids, catechins, coumarins, terpenoids) and the smaller molecules like ascorbic acid (Vitamin C), alpha-tocopherol, beta-carotene, Vitamin-E and their supplements that are usually components of foods (Prochazkova *et al.,* 2011).

2.8.2 Classification of Human Antioxidants According to their Functions

By functions, antioxidant can be classified into: first line defence antioxidants, second line defence antioxidants and third line defence antioxidants.

2.8.2.1 First Line Defence Antioxidants

Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), Glutathione reductase (GR) and some minerals like Selenium, Manganese, Copper and Zinc come under first line defence antioxidants (Khalid *et al.,* 2013).

Catalase (CAT): is an antioxidant enzyme widely distributed in all animal tissues. The enzyme is known to protect the system from highly reactive hydroxyl radicals through hydrogen peroxide decomposition. Depletion of this enzyme may enhance the cellular damage caused by assimilation of superoxide and hydrogen peroxide (Abiodun *et al.,* 2014; Mariam *et al.,* 2015). Among the antioxidant enzymes, catalases are ubiquitous haeme enzymes that are found in aerobic organisms, ranging from bacteria to higher plants and animals (Khalid, 2007). Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Mates *et al*., 1999).

Glutathione peroxidases (GPxs): are members of the family of antioxidant enzymes that scavenge hydrogen peroxide in the presence of reduced glutathione, and seven isoforms having different substrate specificities and tissue distribution have been identified.Glutathione peroxidase is a selenium dependent enzyme that contains a selenium atom incorporated within the selenocysteine residue (Khalid, 2007) which utilizes the reducing equivalents of glutathione to reduce hydrogen peroxide and it may be the main mechanism for protection against the deleterious effects ofhydrogen peroxides.

Superoxide dismutase (SOD): is a group of metallo-enzymes that play a critical role in the first line of defence against oxidative stress caused by free radicals in many organisms. SOD protects cells and cell components against reactive oxygen species (ROS) by catalysing the conversion of oxygen radicals to hydrogen peroxide and molecular oxygen, and thus provides a protective role against oxidative stress (Khalid *et al.*, 2013; Abiodun*et al.,* 2014).

2.8.2.2 Second Line Defence Antioxidants

Reduced glutathione (GSH), Vitamin C, Vitamin E, uric acid, carotenoids, albumin, bilurubin, Vitamin A and flavonoids come under second line defence antioxidants (Marcio and Isabel, 2013).

2.9.2.3 Third Line Defence Antioxidants

Lipase, proteases, DNA repair enzymes, transferases and methionine sulphoxide reductase come under third line defence antioxidants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals/Reagents

All assays kits were purchased from Randox laboratories Ltd. Ardmore, Co. Antrim UKand Reckon Diagnostic Limited, 3/7 Industrial Estate, Garwa Vadodara, India. Chemicals and reagents used were purchased from Sigma Chemical Company St. Louis U.S.A.Some of the chemicals include: Folin Ciocalteu Phenol, gallic Acid, quercetin, Carbon tetrachloride, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich), sodium hydroxide, sodium carbonate, aluminium chloride, sodium nitrate, sodium chloride.

3.1.2 Equipment

Jenway 6405 UV/V Spectrophotometer, Water Bath,Heraeus Labofuge 300 Centrifuge, RS-232C Electronic Weighing Balance, Thomas-Wile Laboratory Mill Model 4, Heamatocrit HAE 3102 reader and Incubator.

3.2 Methods

3.2.1Collection and Identification of Plant Material

Fresh leaves of *Ficus glumosa* were obtained from "*Gwarinpa"* Area Council of Federal Capital Territory-Abuja, Nigeria on 21 June, 2015. The plant leaves were then authenticated at the Herbarium Unit of Biological Science Department, Ahmadu Bello University, Zaria-Nigeria where a voucher specimen numbered 900746 was deposited for further reference.

3.2.2 Animal Management and Care

A total number of 47 apparently healthy *albino* rats of both sexes weighing between 100-150g were obtained from Animal House of Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State. They were separated into males and females in well aerated laboratory cages and acclimatized for a period of 2 weeks in the laboratory condition maintaining light and dark cycle of 12 hours each before the commencement of the experiment. They were fed daily with grower mash from Vital Feeds Company and clean tap water *ad libitum* during this period.

3.2.3 Preparation and Extraction of Plant Material with Methanol

The collected plant leaves were rinsed in clean water and air dried at room temperature under the shade to constant weight. The dried leaves were pulverized into powder using Thomas-Wiley laboratory mill (model 4) before being extracted by cold maceration.

Exactly 500 g of the pulverized plant leaves was then suspended in 2.5 L of absolute methanol and the solution was left standing for 48 hours in large amber bottles with intermittent shaking. At the end of the extraction, the crude methanol extract was filtered using Whatman No. 1 filter paper (1mm mesh size) and then concentrated using water bath maintained at 45°C until dark residue was obtained. The concentrated methanol extract was stored in an air-tight sample container in a refrigerator for further analysis.

The percentage yield was then calculated using the formula:

$$\text{Percentage yield of crude extract} = \frac{\text{weight of extract (g)}}{\text{weight of sample (g)}}\ x\ 100$$

3.2.4 Fractionation of Crude Methanol Extract of *Ficus Glumosa*Leaves

The crude methanol extract (20.13 g) was re-dissolved in 300ml of distilled water and repeatedly partitioned in a separating funnel with 400ml of *n*-hexane for three times with vigorous shaking. At each portioning, the mixture was allowed to stand for 30 minutes to separate into distinct layers of hexane and aqueous. The *n*-hexane fraction was then collected and concentrated using water bath. The aqueous layer was then repeatedly partitioned with 400ml of ethyacetate for three times to obtain ethylacetate fraction. The aqueous layer from the above was then saturated with distilled water and repeatedly partitioned with 400ml of *n*-butanol for three times after which the *n*-butanol fraction and aqueous residue were obtained. The fractions were concentrated using water bath maintained at 45^{o}C until the residues were obtained. The residual fractions were kept in sealed containers and refrigerated at 2-4ºC for further use.

The percentage yield was the calculated using the formula:

$$\text{Percentage yield of fraction} = \frac{\text{weight of fraction (g)}}{\text{weight of crude extract (g)}}\ x\ 100$$

3.2.5 Qualitative Screening of Some Phytochemical Constituents of Methanol Extract of *Ficus Glumosa*Leaves

3.2.5.1 Test for Alkaloids

Meyers Test:Three drops of the reagent were added to 1ml of sample of the extract in a test tube and green precipitate formed indicates the presence alkaloids(Trease and Evans 1983).

3.2.5.2 Test for Cardiac Glycosides

Kella Killiani Test: Exactly0.5g of extract was dissolved in 10ml of glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degree, 1ml of concentrated sulphuric acid was added down the side purple ring colour at the interface indicatesthe presence of cardiac glycosides (Trease and Evans 1983).

3.2.5.3 Test for Saponins

Frothing test: Exactly 0.5g of the extract was dissolved in 10ml of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honey comb formed for more than 30 minutes indicates saponin presence (Trease and Evans, 1983).

3.2.5.4 Test for Flavonoids

Sodium Hydroxide Test: Three drops of aqueous NaOH were added to 5ml of extract, a yellow colouration shows the presence of flavonoid (Trease and Evans, 1983).

3.2.5.5 Test for Tannins

Ferric chloride Test:Exactly 0.5g of extract was dissolved in 10ml of distilled water, and then filtered. Three drops of ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicates hydrolysable tannins and green precipitates indicate the presence of condensed tannin (Trease and Evans, 1983).

3.2.6 Quantitative Screening of Some Key Phytochemical Constituents Present in Fractions of Methanol Extract of *Ficus Glumosa*Leaves

3.2.6.1 Determination of Total Phenolic

Total phenolic content was estimated by Folin Ciocalteu's method as described by Bhalodia *et al.* (2011).

Principle**:**The reaction is based on the reduction of phosphor-wolframate-phosphomolybdate complex by phenolics to a blue reaction product with a maximum absorptionat 765nm which can be measured spectrophotometrically.

Procedure:Exactly 1ml of sample (1 mg/5 ml) and standard gallic acid (0.625, 1.25, 2.5, 5, 10 and 20 µg/ml) were positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was added, mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water. The mixture was incubatedfor 2 hours at room temperature after which intense blue colour was developed. After incubation, absorbance was measured at 750 nm. The blank was performed using reagent blank with solvent. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of solvent fractions of crude methanol leaves extract of *Ficus glumosa* were expressed as mg of gallic acid equivalent weight (GAE)/100 g of dry mass.

3.2.6.2 Determination of Total Flavonoid

Total flavonoid content was measured with the aluminium chloride colorimetric assay described by Pallab *et al.*(2013).

Principle**:** Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the orthodihydroxyl groups in the A or B-ring of flavonoids.

Procedure: Exactly 1ml of sample (1g/5ml) and 1ml standard quercetin solution (10, 20, 30, 40, 50 and 60 µg/ml) were positioned into test tubes and 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution was added into each tube. After 5 minutes, 0.3 ml of 10 % aluminium chloride was added. At 6^{th} minute, 2 ml of 1 M sodium hydroxide was added.

Finally, the volume was made up to 10 ml with distilled water and mixed well. Orange yellowish colour was developed and the absorbance was measured at 510 nm. The blank was performed using distilled water. The calibration curve was plotted using standard quercetin. The data of total flavonoids of solvent fractions of methanol extract of *Ficus glumosa*leaves were expressed as mg of quercetin equivalents/100 g of dry mass.

3.2.6.3 Determination of Ascorbic Acid Content

Ascorbic acid was determined using the method described by Barros *et al.,* (2007).

Principle**:** Ascorbic acid reacts with 2, 6-dichlorophenolindophenol (DCPIP) (blue colour) in acidic medium is reduced to (DCPIPH,pink) that is further reduced to a colourless compound ($DCPIPH_2$) by ascorbic acid. The absorbance is read spectrophotometrically at 515 nm.

Procedure: One gram of each fraction was diluted with 10 ml of 0.5% oxalic acid and the mixture was shaken and left for 20 minutes at room temperature and was filtered through Whatman No. 4 filter paper. Precisely 1 ml of the filtrate was mixed with 9 mL of 0.1 M of 2, 6-dichlorophenolindophenol reagent. A reagent blank using distilled H_2O instead of sample was prepared. The absorbance was read within 30 minutes at 515 nm against the prepared blank. This test was carried out in triplicates. The ascorbic acid content was calculated using the calibration curve, prepared from standard L-ascorbic acid (0.65, 1.25, 2.5, 5 and 10 mg/ml). The data obtained were expressed as mg L-ascorbic acid equivalent per gram of dry matter.

3.2.7 Determination of Antioxidant Activityof Fractions of Methanol Extract of *Ficus Glumosa* Leaves

The antioxidant activity of fractions of methanol extract of the plant was assayed by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method described by Karadag *et al.,* (2009).

Principle**:**1,1-Diphenyl-2-picrylhydrazyl (DPPH) contains an odd electron in its structure. Its deep purple colour is reduced to a colourlesscompound, 2,2-diphenyl-1-picryhydrazine when it reacts with an antioxidant, which can donate a hydrogen atom or an electron to it. The change in colour was measured spectrophotometrically at 517 nm using a UV/Visible light spectrophotometer.

Procedure: The assay mixture contained 2ml of 1.0 mM DPPH radical solution prepared in methanol and 1ml of standard or extract solution of different concentrations (10 – 500 μg/ml). The solution was rapidly mixed and incubated in dark at 37^{o}C for 20 minutes. The decrease in absorbance of each solution was measured at 517nm using spectrophotometer. Ascorbic acid was used as positive control while 2 ml of 1.0 mM DPPH radical solution with 1ml ethanol was taken as blank.

The percentage of radical scavenging (%) was calculated by:

% Free Radical Scavenging Activity = $A_c – A_s/A_c$ x 100

Where, A_c = Absorbance of control at 517nm

A_s = Absorbance of sample at 517nm

The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percentage inhibitions plotted against the respective concentrations.

3.2.8Acute Toxicity Study of *n*-Butanol Fraction of Methanol Extract of *F. Glumosa* Leaves

The median lethal dose (LD_{50}) of *n*-butanol fraction of methanol extract of *Ficus glumosa*leaves was conducted in order to select suitable safe doses for the evaluation of effects of the*n*-butanol fraction. This was done using the method described by Lorke (1983). In the initial phase, rats were divided into 3 groups of 3 rats each and were treated with 10mg, 100mg and 1000mg of *n*-butanol fraction per kg body weight orally. They were observed for

24 hours for signs of toxicity, including behavioural changes and death. In the final phase, 3 rats were divided into 3 groups of one rat each, and were treated with *n*-butanol fraction based on the findings in the first phase. Based on the survival from phase one, 3 rats were separately treated with 1600, 2900 and 5000 mg/kg body weight of the *n*-butanol extract fraction in the second phase respectively, the number of death within 24 hours were recorded. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of the consecutive doses with 0 and 100% survival rates were recorded.

3.2.9 Experimental Design to Assess Ameliorative Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves Against CCl_4-Induced Liver Toxicity and Oxidative Stress

3.2.9.1 Animal Grouping

A total of 35 albino rats were used. The rats were divided into 7 groups of 5 animals each:

Group 1: Normal control rats received vital feed and tap water only. This served as the normal control group.

Group 2: Rats were treated with olive oil and served as vehicle control group.

Group 3: Rats were treated with 1ml/kg body weight 50% Carbon tetrachloride (CCl_4) in olive oil. This served as the CCl_4 control group without extract or standard drug treatment.

Group 4: Rats were treated with 1ml/kg body weight 50% CCl_4 in olive oil + 100mg/kg body weight/day *n*-butanol fraction.

Group 5: Rats were treated with 1ml/kg body weight 50% CCl_4 in olive oil + 300mg/kg body weight/day *n*-butanol fraction.

Group 6: Rats were treated with 1ml/kg body weight 50% CCl_4 in olive oil + 500mg/kg body weight/day *n*-butanol fraction.

Group 7: Rats were treated with 1m/kg body weight 50% CCl_4 in olive oil + 100 mg/kg body weight/daysilymarin as standard drug reference.

3.2.9.2 Hepatotoxicity and Oxidative Stress Induction using $CCl_4$and Treatment with n-Butanol Fraction of Methanol Extract of Ficus Glumosa Leaves

The animals were pre-treated on the first day of the experiment with 1 ml/kg body weight from 50% solution of CCl_4 in olive oil (IP) followed by oral administration of the extract after 24 hour of intoxication with CCl_4. The administration of the *n*-butanol fraction was continued for a period of 21 days with once weekly challenge with 1ml/kg body weight 50% solution of CCl_4. The animals were fasted for 24 hours after the last administration of the extract and sacrificed at the end of the experiment for sample collection and subsequent analysis (Akram *et al.*, 2012).

3.2.10 Collection and Preparation of Animal Samples

3.2.10.1 Collection and Preparation of Sera Samples

At the end of 21 days of treatment, the animals were sacrificed by decapitation using chloroform anaesthesia and blood samples were collected from the throat in plain bottles (for biochemical parameters) and in EDTA bottles for haematological analysis. The Blood samples collected in plain tubes were allowed to clot and the sera were separated by centrifugation using Labofuge 300 centrifuge (Heraeus) at 3000 rpm for 10 minutes. The sera collected were then subjected to biochemical analysis.

3.2.10.2 Collection of Liver and Kidney for Homogenization

Immediately after the blood was collected, liver and kidneys were quickly excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed (so as to calculate the relative weight) and were kept on ice. One gram of the liver and kidney each were taken for tissue homogenatepreparation while the rest of the organs were placed in freshly prepared 10% formalin for histopathological studies. Exactly 1g of the organ each was homogenized in 10 ml of buffer (50 mM potassium phosphate buffer, pH 7.4) using pestle and mortar. The homogenate was then centrifuged at 4000 rpm (2700 x g) for 15 minutes and the supernatant was collected using Pasteur pipette. The percentage change in organ weight of each of the animals was calculated as follows:

$$\text{Percentage change in organ weight} = \frac{\text{organ weight (g)}}{\text{animal weight (g)}}\ x\ 100$$

3.2.11Toxicological Studies on Liver Function of Experimental Animals

3.2.11.1 Assessment of Aspartate Aminotransferase (AST) Activity

AST activity was determined by the method described by Amador and Wacker (1962).

Principle:In this reaction L-Aspartate and α-Ketoglutarate react in the presence of AST in the sample to yield oxaloacetate and L-glutamate. The oxaloacetate is reduced by malate dehydrogenase to yield L-malate with the oxidation of NADH to NAD^+. The reaction is monitored by measurement of the decrease in the absorbance of NADH at 340nm. The rate of reduction in absorbance is proportional to AST activity in the sample.

Procedure: To 1 ml of reagent added to all required test tubes, 0.05 ml of the sample was added to the sample test tube and none to the blank. It was incubated at room temperature for 20 min, mixed immediately and first absorbance of test was read exactly at 1 minute and thereafter at 30, 60, 90 and 120 seconds at 340 nm. The mean change in absorbance per minute was determined and the test results were calculated.

Serum AST activity (IU/L) = ΔA /min × F.

ΔA = Change in absorbance per minute

F = 3376 (Based on the millimolar extinction coefficient of NADH at 340 nm)

3.2.11.2 Assessment of alanine aminotransferase (ALT) activity

ALT activity was determined by method described by Amador and Wacker (1962).

Principle: In this reaction, L-alanine and α-ketoglutarate react in the presence ALT in the sample to yield Pyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate with oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance at 340 nm. The rate of reduction is proportional to ALT activity in the sample.

Procedure:To 1 ml of reagent added to all required test tubes, 0.05 ml of the sample was added to the test sample tube test and none to the blank. It was incubated at room temperature for 20 min, it was mixed immediately and first absorbance of test was read at exactly 1 minute and thereafter at 30, 60, 90 and 120 seconds at 340 nm. The mean change in absorbance per minute was determined and test results were calculated.

Serum ALT activity (IU/L) = Δ**A**/min × F.

ΔA = Change in absorbance per minute

F = 3376 (Based on the millimolar extinction coefficient of NADH at 340 nm)

3.2.11.3 Assessment of Alkaline Phosphatase (ALP) Activity

Serum activity of alkaline phosphatase (ALP) was determined by the method described by Haussament, (1977).

Principle: P-nitrophenylphosphate + $H_2O \xrightarrow{ALP}$ phosphate + p-nitrophenol (405 nm)

Alkaline Phosphatase in a sample hydrolyses paranitrophenyl phosphate into paranitrophenol and phosphate, in the presence of magnesium ions. The rate of increase in absorbance of the reaction mixture at 405 nm and 37^0C due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity.

Procedure: Reagent (1 ml) containing diethanolamine buffer, magnesium chloride and substrate (P-nitrophenylphosphate) was added into a clean test tube and incubated at 37^0C followed by the addition of 0.02 ml of sample. This was mixed thoroughly and immediately absorbance of test was read exactly at 30, 60, 90 and 120 seconds at 405nm against the reference blank (distilled water).The mean change in absorbance per minute was determined and the test results were calculated. Calculation:

The ALP activity was calculated using the following formulae:

Serum Alkaline phosphatase Activity (IU/L) = Δ**A**/min x F.

ΔA = Change in absorbance per minute

F = 2713 (calculated on the basis of molar extinction coefficient for paranitrophenol and ratio of total assay to sample volume)

3.2.11.4 Determination of Serum Bilirubin Concentration

The serum total and direct bilirubin concentrations were determined by the method Jendrassik and Gróf (1938).

Principle: Bilirubin is estimated by reacting it with diazotised sulfanilic acid obtained from sodium nitrite and sulfanilic acid solutions. Bilirubin when reacted with diazotised sulfanilic acid forms a pink coloured azo-compound that is measured at 546 nm. The unconjugated or free bilirubin takes longer time to react and requires caffeine as accelerator. The indirect bilirubin is calculated from the difference between the total and direct bilirubin.

Procedure for total bilirubin**:**Sample (0.05 ml) was pipetted into both the standard and test tubes. Then to the standard tube, 0.1 ml of 2-bilirubin solution was added followed by the addition of 1.0 ml of 3-bilirubin solution. Also, to the test tube, 0.1 ml of working reagent was added followed by 1.0 ml of 3-bilirubin solution. The reaction was incubated at room temperature for 5 minutes and absorbance was read at 546 nm against sample blank.

Procedure for direct bilirubin**:**Sample (0.05 ml) was pipette into both the standard and test tubes. Then to the standard tubes, 1.0 ml of normal saline was added and followed by the addition of 0.1 ml of 2-bilirubin solution. Also, 1.0 ml of normal saline was added to the test tubes and followed by the addition of 0.1 ml of working reagent. The reaction was incubated at room temperature for 3 minutes and absorbance was read at 546 nm against sample blank.

Calculation: Serum Bilirubin (mg/dl) = (Absorbance of sample-Absorbance of sample blank) x F

F =26.312 (Molar extinction coefficient of diazotised sulfanilic).

3.2.11.5 Determination of Total Protein Level

Total protein was determined colorimetrically according to the method described by Fine (1935).

Principle: In an alkaline medium, protein reacts with the copper in the biuret reagent causing an increase in absorbance. The increase in the absorbance, at 540nm due to formation of the coloured complex (alkaline-copper-protein) is directly proportional to the concentration of protein.

Procedure:Biuret reagent (2.5 ml) was added to all the required test tubes (sample blank, standard and test sample). Also, 0.05 ml of the sample was added to the test sample tubes and 0.05 ml of the standard reagent was added to the standard test tube. It was mixed well and allowed to stand at room temperature for 10 minutes. The absorbance of the test sample and standard were read at 540 nm against sample blank. The concentration of the sample was calculated using the formula.

$$\text{Total Protein Conc. (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}}\ x\ \text{Concentrationof Standard}$$

3.2.11.6 Determination of Albumin Level

The serum albumin was determined by the method of Doumas *et al*.(1971).

Principle: In an acidic medium, albumin binds with bromocresol green causing a shift in the absorption spectra of the yellow BCG dye. The blue green colour formed is directly proportional to the albumin present when measured at 630 nm.

Procedure:Bromocresol green reagent (2.5 ml) was added to three clean test tubes labelled test sample, standard and sample blank. Also, 0.01ml of the sample was added to the test sample and 0.01 ml of the standard reagent was added to the standard test tube respectively. The mixtures in each of the test tubes were mixed well and allowed to stand at room temperature for 10 minutes. The absorbance of the test sample and of the standard was read after 10 mins at 630 nm against the sample blank.

Calculation:

$$\text{Albumin Conc. (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}}\ x\ \text{Concentrationof Standard}$$

3.2.12Toxicological Studies on Renal Function of Experimental Animals

3.2.12.1 Determination of Serum Urea Concentration

This was assessed using the method described by Fawcett and Scout (1960).

Principle**:** Urease breaks down urea into ammonia and carbondioxide. In alkaline medium, ammonia reacts with hypochlorite and salicylate to form dicarboxyindophenol, a coloured compound. The reaction is catalysed by sodium nitroprusside. The intensity of colour produced is measured spectrophotometrically at 578 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{salicylate} \longrightarrow \text{dicarboxyindophenols (blue compound)}$$

Procedure:Reagent (1 ml) containing sodium nitropusside and urease was added into three clean test tubes labelled as test sample, standard and reagent blank containing 0.01 ml sample, 0.01 ml standard reagent and 0.01 ml distilled water respectively. The content in each of the test tube was mixed and incubated at room temperature (25-30^{o}C) for 10 minutes. The absorbance of the test sample and standard were read against reagent blank at 578 nm.

Calculation:The serum urea concentration was calculated using the formula below;

$$\text{Urea Conc. (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}}\ x\ \text{Concentrationof Standard}$$

BUN Concentration (mg/dl) = 0.467 x Urea Concentration (mg/dl).

3.2.12.2 Determination of Serum Creatinine Concentration

The colorimetric method was used to determined serum creatinine concentration according to Bertels and Bohmer (1973).

Principle: Creatinine present in the serum reacts with alkaline picrate to form a coloured complex. The rate of formation of coloured complex is directly proportional to creatinine concentration. This rate of reaction (intensity of orange colour produced) is measured colorimetrically at 510 nm and is compared with that of the standard.

$$\text{Creatinine + Picric acid (Yellow)} \xrightarrow[\text{(Alkaline medium)}]{\text{NaOH}} \text{Creatinine picrate (510 nm) (Orange)}$$

Procedure:Working reagent (1 ml) containing picric acid and sodium hydroxide was added into two clean test tubes labelled sample test and standard, containing 0.1ml of test sample and 0.1ml of standard solution. The content in each test tube was mixed and after 20 seconds, the absorbance of the standard (ST1) and test sample (TS1) was read at 510 nm. Exactly 80 seconds later, absorbance for (ST2) and (TS2) of the standard and sample were read at 510 nm against distilled water (blank).

Calculation: The Concentration of creatinine in serum (mg/dl) was calculated using the formula below:

$$\text{Creatinine Conc. (mg/dl)} = \frac{\text{TS2} - \text{TS1}}{\text{ST2} - \text{ST1}}\ x \text{ Concentration of Standard}$$

(ST= Standard, TS= Test Sample)

3.2.12.3 Estimation of Serum Sodium, Potassium and Chloride ions

A flame photometer Model 143, equipped with an automatic diluter Model 144 (ratio of the dilution of 200:1) (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A.) was used. The calibration of the flame photometer was performed with twice distilled water and a standard having a Na^+ concentration of 140 mequiv/l and a K^+ concentration of 5 mequiv/l (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A). The stability of the instrument was checked with the standard solution after each measurement of a sample.

3.2.13 Determination of *In Vivo* Antioxidant Status in Liver and Kidney of Experimental Animals

3.2.13.1 Estimation of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was measured using the method described by Martin *et al*., 1987.

Principle:Auto-oxidation of haematoxylin (with increase in absorbance at 560 nm) is inhibited by SOD activity at the assay pH 7.8; the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample was determined by measuring the amount of haematin formed at 560 nm.

O_2 + HTH_2 $\xrightarrow{\text{SOD}}$ H_20_2 + HT (Abs 560nm)

(Haematoxylin) (Haematin)

Procedure:Assay buffer (phosphate buffer 0.05M, pH 7.8) (920 uL) was added to a clean test tube followed by the addition of 40μL of sample (tissue homogenate) which was indicated as sample test. A reagent test (sample blank) was also prepared by adding 40 μL of assay buffer (phosphate buffer 0.05M, pH 7.8) to another clean test tube.The mixtures were shaken and incubated for 2 minutes at room temperature. Also, 40 μL of haematoxylin was added to both sample test and reagent test tubes (sample blank) and were mixed quickly to start the auto-oxidation reaction. Following the addition of 40 μL of haematoxylin, absorbance of the sample test and reagent test was read at 560 nm every 30 seconds for 5 minutes against distilled water.

Calculation:

SOD activity was determined by measuring the ratios of auto-oxidation rates in the presence and absence of the sample. SOD activity in the sample was calculated thus;

Absorbance $_{\text{Reagent test}}$ (A_R) = Absorbance $_{\text{Reagent test 2}}$ − Absorbance $_{\text{Reagent test 1}}$

Absorbance $_{\text{sample test}}$ (A_S) = Absorbance $_{\text{sample test 2}}$ − Absorbance $_{\text{sample test 1}}$

% SOD inhibition = $[1 - A_S/A_R]x100$

SOD activity (U/ml) = $[1 - A_S/A_R]x100 \times 1.25$

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the auto-oxidation of haematoxylin to haematin in 1 minute.

3.2.13.2 Estimation of Catalase (CAT) Activity

Catalase activity was determined using the method described by Aebi and Bergmeyer (1983).

Principle:Catalase scavenges hydrogen peroxide (H_2O_2) converting it to water and molecular oxygen.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

(Absorbance at 240 nm) (no Absorbance at 240 nm)

The activity of catalase in the sample was determined by following the rate of decrease in absorbance at 240 nm.

Procedure**:** Assay buffer [50 Mm potassium phosphate buffer (pH 7.0)] (1000 µL) was added to a cuvette and used to zero the spectrophotometer at a wavelength of 240 nm. Also, 950 µL of working assay buffer [490 µL of50 Mm potassium phosphate buffer (pH 7.0) and 460 µL of 30 Mm hydrogen peroxide (H_2O_2)] and 50 µL of sample (tissue homogenate) were pipetted to another clean cuvette, mixed quickly. Also, a catalase standard was prepared by adding 50 µL of diluted catalase standard to 950 µL of working assay buffer. The decomposition rate of H_2O_2 was measured at 240 nm every 1minute for 5 minutes. Catalase activity was determined and expressed as (U/ml) from the decomposition rate given as (ΔA240nm/min) of the sample.

$\Delta A240nm/min$= Change in absorbance per minute.

Catalase (U/ml) = ($\Delta A240nm/min$) / Volume of reaction mixture

3.2.13.3 Estimation of Glutathione Peroxidase (GPx) Activity

Glutathione Peroxidase Assay is an adaptation of the method of Paglia and Valentine(1967).

Principle**:** Glutathione Peroxidase catalyses the reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming $NADP^+$ (resulting in decreased absorbance at 340 nm) and recycling the GSH. Because Glutathione Peroxidase is the reaction rate limiting enzyme, the decrease in absorbance at 340 nm is directly proportional to the Glutathione Peroxidase activity.

Procedure: All reagents were brought to room temperature and samples (tissue homogenate) were placed on ice. The NADPH reagent (β-nicotinamide adenine dinucleatide phosphate and GSH reduced) was reconstituted with NADPH diluent (glutathione reductase in buffer with stabilizer and 4mM NaN_3) and was labelled as working NADPH. Exactly 50 µL of sample was added to a clean test tube followed by the addition of 50 µL working NADPH. Also, 50 µL of working H_2O_2 (0.3 ml of 3% H_2O_2 diluted to 10 ml with assay buffer) was added to the sample test tube and was allowed to equilibrate for 1 minute. Sample blank tube was prepared by replacing the sample with 50 µL of distilled water. The mixtures in both tubes were transferred to cuvettes and absorbance was read at 340nm for 5 minutes with 30 seconds recording intervals against sample blank.

Calculations:Glutathione Peroxidase activity was calculated from the net rate and expressed as (U/ml).

$$\text{GPx} = \frac{2\ (\text{mRates} - \text{mRateb})150}{6.22 \text{ x } 50}$$

Where;

$mRate_s$ = 1000 x ΔA_{340}/min of sample

$mRate_b = 1000 \times \Delta A_{340}$/min of blank

6.22 = NADPH 340 nm millimolar absorption coefficient at 1 cm path length.

150 µL = Volume of reaction mixture

50 µL = Volume of sample

2= Correction factor for 2 moles GSH oxidized to 1 mole GSSG per mole NADPH oxidized.

3.2.13.4 Estimation of Thiobarbituric Acid Reactive Substance (TBARS)

A thiobarbituric acid reactive substance (TBARS) in the tissues was estimated using the method described by Fraga *et al.*(1988).

Principle: The formation of malondialdehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation.At low pH of 2-3 and high temperature (60^0C), malondialdehyde (MDA) binds thiobarbituric acid (TBA) to form a pink complex (MDA-TBA) adduct which absorbs maximally at 532 nm.

Procedure:Tissue homogenate (sample) (250 µL), 10 µL of BHT reagent (butylated hydroxytoluene in ethanol), 250 µL acid reagent (1M phosphoric acid) and 250 µL of TBA reagent (2-thiobarbituric acid reconstituted with 10.5 ml distil water) were added to a clean sample centrifuge tubes, mixed vigorously. A sample blank test was prepared by replacing the sample with 250 µL of distilled water and the mixture in both tubes were incubated for 60 minutes at a temperature of 60^0C in a water bath, cooled and centrifuged at 10,000 xg for 3 minutes. The reaction mixture in both tubes was transferred to cuvettes and the absorbance of the pink colour produced was read at 532 nm for 5 minute against sample blank.

Calculations:The concentration of TBARS is expressed in terms of Malondialdehyde (MDA) in µM.

Molar extinction of MDA = $1.56 \times 10^5 M^{-1}cm^{-1}$

MDA concentration = Absorbance / $1.56 \times 10^5 M^{-1}cm^{-1}$

3.2.14. Estimation of Some Lipid Profile of Experimental Animals

3.2.14.1 Determination of Serum Total Cholesterol (TC)

The serum total cholesterol was quantified by the method described by Stein (1987).

Assay principal:The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and perioxidase.

Cholesterol ester + H_2O $\xrightarrow{\text{esterase}}$ Fatty acids

Cholesterol +O_2 $\xrightarrow{\text{oxidase}}$ Cholestene-3-one +H_2O_2

$2H_2O_2$ + phenol + 4-Aminoantipyrine $\xrightarrow{\text{peroxidase}}$ Quinoneimine + $4H_2O$

Procedure:Exactly 1000µl of the cholesterol reagent which is made up of 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and buffer was added into a clean test tube containing 10 µl of serum, mixed and incubated for 5 minutes at 37^oC. The absorbance was read against the reagent blank at 500nm within 60 minutes.

Calculation:

Using the standard, the concentration of cholesterol in the sample is given as:

Conc. of cholesterol (mg/dl) = ΔA_{sample} / $\Delta A_{standard}$ X Conc. of Standard

ΔA_{sample} = Change in absorbance of the sample

$\Delta A_{standard}$ =Change inabsorbance of the standard

3.2.14.2 Determination of Serum Triacylglycerol (TAG)

The serum triacylglycerol level was estimated by enzymatic method described by Stein (1987).

Assay principle:The triacylglycerol is determined after enzymatic hydrolysis with lipases. The indicator quinoneimine is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Triacylglycerol + H_2O $\xrightarrow{\text{Lipases}}$ Glycerol + Fatty acids

Glycerol + ATP Glycerol kinase $\longrightarrow$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 GPO $\longrightarrow$ Dihydroxyacetone + Phosphate + H_2O_2

2H2O2 + 4-Aminophenazone + 4-Chlorophenol peroxidase $\longrightarrow$ Quinoneimine + $4H_2O$

Procedure:Exactly 1000 µl of the cholesterol reagent which is made up of 4-aminophenazone, peroxidase, glycerol kinase, Glycerol-3-phosphate oxidase and buffer was added into a clean test tube containing 10 µl of serum, mixed and incubated for 5 minutes at 37^oC. The absorbance was read against the reagent blank at 500nm.

Calculation:

Conc. of Triacylglycerol (mg/dl) = ΔA_{sample} / $\Delta A_{standard}$ X Conc. of Standard

ΔA_{sample} = Change in absorbance of the sample

$\Delta A_{standard}$ = Change in absorbance of the standard

3.2.14.3.Determination of Serum High Density Lipoprotein-cholesterol (HDL-c)

The serum levels of HDL-c were determined by enzymatic method described by Stein (1987).

Assay principle:

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

Procedure:Exactly 0.5 µl of reagent A made which is up of phosphotungstate and magnesium chloride was added into a clean test tube containing 0.2ml of serum, mixed thoroughly and allowed to stand for 10 minutes at room temperature, centrifuged at 4000 rpm for 10 minutes and the supernanant was collected. Exactly 0.1ml of reagent B made up of 4-aminoantipyrine, sodium cholate and dichlorophenolsulfonate was added into a clean test tube containing 50 µl of sample supernatant, mixed thoroughly and incubated for 30 minutes at room temperature. The absorbance was read against the reagent blank at 500nm within 60 minutes.

Calculation:

HDL-c += ΔA_{sample} / $\Delta A_{standard}$ X Conc. of Standard

ΔA_{sample} = Change in absorbance of the sample

$\Delta A_{standard =}$ Change in absorbance of the standard

3.2.15 Assessment of some haematological parameters of experimental animals

3.2.15.1 Determination of Packed Cell Volume (PCV)

The PCV is the volume of red blood cells (RBC) expressed as a fraction of the total volume of the blood. The microhaematocrit method was used (Cheesbrough, 2000).

Principle: The red blood cells are heavier than plasma with specific gravity of 1090 and 1030 respectively. When blood is placed in a capillary tube and centrifuge, they settle and packed because of the centrifugal force acting on them. The volume occupied by the cells is measured with a haematocrit reader relative to the volume of the whole blood.

Procedure: Blood sample from the rats were filled into heparinized capillary tube after which one end of the tube was sealed by flaming. It was then centrifuged at a speed of 7000 rpm for 5 minutes. The PCV was estimated using a microhaematocrit reader and expressed as percentage erythrocytes that the blood contain.

3.2.15.2 Determination of Haemoglobin Concentration (Hb)

Haemoglobin concentration (Hb) was determined using the cyanmethaemoglobin of Alexander and Griffins (1993a and 1993b) respectively.

Principle:Blood is mixed with Drabkin's solution, a solution that contains ferricyanide and cyanide. The ferricyanide oxidizes the iron in the haemoglobin, thereby changing haemoglobin to methaemoglobin. Methaemoglobin then unites with the cyanide to form cyanmethaemoglobin. Cyanmethaemoglobin produces a brownish-coloured solution which is then measured in a spectrophotometer at 540 nm. The colour relates to the concentration of haemoglobin in the blood.

Procedure**:** Sample solutions and standard solutions were prepared as follows.

Blank: Exactly 5000 µl of Drabkin reagent was mixed with 20 µl of distilled water

Standard: Exactly 5000 µl of Drabkin reagent was mixed with 20 µl of standard solution of haemoglobin.

Test sample: Exactly 5000 µl of Drabkin reagent was mixed with 20 µl of blood.

The concentration of haemoglobin was marked with Drabkin's method, with the use of a spectrophotometer. Once Drabkin reagent was mixed with the blood, the solution was incubated at room temperature for the duration of 5 mins and absorbance was measured at 540 nm against distilled water.

The concentration of haemoglobin was calculated according to the following formula:

$$\text{Hb concentration (g/dl)} = \frac{\text{absorbance of tested sample}}{\text{absorbance of standard}} \text{ X concentration of standard in g/dl}$$

3.2.15.3 Estimation of White Blood Count (WBC)

The total white blood count was determined using counting chamber method described by W.H.O, (2000).

Procedure: 20 µl of the blood sample was added to 0.4ml of diluting fluid which consists of 2% acetic acid lightly coloured with 1% crystal violet (1:21 dilution of the blood). The counter chamber was then filled with the mixture from above and allowed to stand for 3-5 minutes after which it was placed on a microscope stage and observed using X25 objective to count number of cells seen a sufficient number of $1mm^2$ areas to obtain at least 100 cells.

Calculations:

Cell count (/L) = N x (D/D) x 10 x 10^6

Where: N = total number of cell counted; D = dilution of blood; A = total area counted (in mm^2); 10 = factor to convert area to volume (in µl); 10^6 = factor to convert count per µl to litre.

3.2.16 Histological study of liver and kidney of experimental animals

A portion of the liver and kidneys of the animals were cut into two to three pieces and fixed in 10% formalin (Lillie, 1965). The paraffin sections were prepared and stained with haematoxylin and eosin. The thin sections of liver and kidneys were made into permanent slides and examined under high (X250) resolution microscope with photographic facility and photomicrographs were taken.

3.3 Statistical Analysis

The data were analysed by the analysis of variance (ANOVA) using SPSS program (version 20 SPSS Inc., Chicago, IL, USA). The differences in parameters between the various animal groups were compared using the Bonferroni multiple comparison test (post-hoc test). The results were expressed as mean ± standard deviation (SD). P value less than 0.05 was considered as significant ($P < 0.05$). Results were presented in table, charts and graphs using MICROSOFT WORD and EXCEL.

CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary Studies on Methanol Extract of *Ficus glumosa* Leaves and its Fractions

4.1.1 The Percentage yield of Crude Methanol Extract of *Ficus Glumosa* Leaves and its Fractions

The Percentage yield following the methanol extraction of *Ficus glumosa* leaves and the various fractions is shown in Table 4.1. The percentage yield % (w/w) of the methanol crude extract was 4.03 % (w/w) while that of aqueous, *n*-butanol, ethylacetate and *n*-hexane were 21.31, 11.73, 6.51 and 4.07 % (w/w) respectively.

4.1.2 Some Qualitative Phytochemical Constituents of Methanol Extract of *Ficus Glumosa* Leaves

Qualitative phytochemical constituents of methanol extract of *Ficus glumosa* leaves is presented in Table 4.2. There was presence of flavonoid, alkaloid, tannin, saponin but absence of cardiac glycoside in the crude methanol extract.

4.1.3 Some Quantitative Phytochemical Constituents of Fractions of Methanol Extract of *Ficus Glumosa* Leaves

Table 4.3 shows the concentrations of total phenol, total flavonoid and ascorbic acid of various solvent fractions of methanol extract of *Ficus glumosa* leaves. The *n*-butanol fraction has higher concentrations of total phenol and flavonoid [9.76±0.63 (mg/100g) Gallic Acid Equivalent and 5.27±0.23 (mg/100g) Quercetin Equivalent respectively] as compared to aqueous, *n*-hexane and ethylacetate fractions. However, the aqueous fraction was found to have the highest concentration of ascorbic acid (0.387±0.08 mg/ml) as expressed in terms of L-Ascorbic Acid Equivalent.

4.1.4 Antioxidant Activity (by DPPH) of Fractions of Methanol Extract of *Ficus Glumosa* Leaves

The free radical scavenging ability of the fractions on DPPH was investigated and the % inhibition values at different concentrations of *n*-hexane, ethylacetate, *n*-butanol and aqueous residue of methanol extract of *Ficus glumosa* leaves were used to determine the IC_{50} values presented in Table 4.4. There was an increasing IC_{50}value in the order of *n*-butanol fraction (0.41±0.07 mg/ml)< aqueous residue (0.71±0.05 mg/ml) < ethylacetate fraction (0.93±0.13 mg/ml) <*n*-hexane fraction (2.77±0.49 mg/ml). The *n*-butanol fraction with the lowest IC_{50} value (0.41±0.07 mg/ml) was then adopted as the fraction with the most active antioxidant potential.

4.1.5. Acute Toxicity Study of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves

Table 4.5 shows the behavioural change of *albino* rats when performing LD_{50} of *n*-butanol fraction of methanol extract of *Ficus glumosa*leaves. It was obvious that most behaviours stated on the table remained normal at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight, except for the activity that slightly increased at the higher doses of 2900 and 5000 mg/kg body weight respectively.

The Median Lethal Dose (LD_{50}) of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves on the tested *albino* rats is described in Table 4.6. There was no death recorded at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight respectively. The LD_{50} of *n*-butanol fraction was taken to be >5000 mg/kg body weight.

Table 4.1: Percentage Yield of Crude Methanol Extract of *Ficus glumosa* Leaves and its Fractions

Extract	Percentage Yield % (w/w)
Crude Methanol	4.03
Aqueous	21.31
n-Butanol	11.73
Ethylacetate	6.51
n-Hexane	4.07

Table 4.2: Some Qualitative Phytochemical Constituents of Methanol Extract of *Ficus glumosa* Leaves

Phytochemical	Status
Flavonoid	+++
Alkaloid	++
Tannin	++
Saponin	+
Cardiac glycoside	-

+++ = Highly present, ++ = moderately present; += low, - = absent

Table 4.3: Some Qualitative Phytochemical Constituents of Solvent Fractions of Methanol Extract of *Ficus glumosa* Leaves

Solvent	Total phenol GAE (mg/100g)	Total flavonoid QCE (mg/100g)	Ascorbic acid LAA (mg/ml)
n-Butanol	9.76 ± 0.63^{a}	$5.27 \pm 0.23b^{a}$	0.359 ± 0.03^{ab}
Ethylacetate	4.73 ± 0.20^{b}	2.19 ± 0.42^{b}	0.202 ± 0.07^{a}
Aqueous	2.60 ± 0.24^{c}	1.46 ± 0.50^{b}	0.387 ± 0.08^{b}
n-Hexane	0.20 ± 0.15^{d}	0.12 ± 0.09^{c}	0.032 ± 0.03^{c}

n=3; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at $P<0.05$.
GAE = Gallic Acid Equivalent, QCE = Quercetin Equivalent, LAA = L-Ascorbic Acid

Table 4.4: DPPH Scavenging Activity of Fractions of Methanol Extract of *Ficus glumosa* Leaves (IC_{50})

Methanol Extract fraction	DPPH Scavenging Activity (IC_{50}) mg/ml
n-Butanol	0.41 ± 0.07^{a}
Aqueous	0.71 ± 0.05^{a}
Ethylacetate	0.93 ± 0.13^{a}
n-Hexane	2.77 ± 0.49^{b}

n=3; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at P<0.05.
DPPH = 1, 1-Diphenyl-2-picrylhydrazyl; IC_{50} = 50% Inhibitory Concentration

Table 4.5: Behavioural Changes of Albino Rats following Administration of *n*-Butanol Fraction of Methanolic Leaf extract of *Ficus glumosa*

Parameter	Dose (mg/kg/bw)					
	10	100	1000	1600	2900	5000
Social Interaction	+	+	+	+	+	+
Activity	+	+	+	+	+ +	+ +
Aggressiveness	+	+	+	+	+	+
Reaction to Noise	+	+	+	+	+	+
Reaction to Touch	+	+	+	+	+	+
State of Tail	+	+	+	+	+	+
State of Excrement	G	G	G	G	G	G

+ = Normal; ++ = slightly increased; G = granular

Table 4.6: Median Lethal Dose (LD_{50}) of *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves after 24 hour

	Dose (mg/kg/bw)	Number of Rat	Mortality	% Lethality
Phase I	10	3	0/3	0
	100	3	0/3	0
	1000	3	0/3	0
Phase II	1600	1	0/1	0
	2900	1	0/1	0
	5000	1	0/1	0

LD_{50}>5000 mg/kg Body Weight

4.2 Toxicological Studies of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves on Liver and Kidney

4.2.1 Percentage Change in Organ Weight of CCl_4-induced Liver Damaged *Albino* Rats

Table 4.7: presents the percentage change in absolute and relative weight of liver and kidney of CCl_4-induced liver damaged *albino* rats treated orally with *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves for a period of 21 days. There was a significant increase (P<0.05) in both the absolute and percentage relative weights of liver and kidney in the CCl_4-induced untreated rats as compared to CCl_4-induced rats that were treated with *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves and silymarin that shows no significant difference (P>0.05) between the normal group (non-induced).

4.2.2 Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves on Liver Biomarkers of Experimental Animals

There was a significant (*P*<0.05) increase in the concentrations of liver marker enzymes (ALT, AST and ALP) as well as direct bilirubin (DB) and total bilirubin (TB) concentrations in the CCl_4-induced untreated group as compared with the normal control group in Table 4.8. However, rats treated with 300 and 500 mg/kg body weight *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves showed a significant (P<0.05) reduction of these indices comparable to 100 mg/kg body weight silymarin and normal control groups while the effect of 100 mg/kg body weight of the extract produced a significant (P<0.05) difference as compared to normal rats and silymarin treated rats even though, the indices were significantly (P<0.05) lower as compared to the untreated rats.In another evaluation, all the doses of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves significantly (*P*<0.05) increased the serum total protein and albumin in rats when compare with the untreated induced control group (Figure 4.1and 4.2).

Table 4.7: Percentage Change in Organ Weight of CCl_4-iduced Liver Damaged *Albino* Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

Group	Liver		Kidney	
	Absolute weight(g)	% Relative weight	Absolute weight(g)	% Relative weight
Normal control	4.60±0.32[a]	2.76±0.39[a]	1.002±0.11[a]	0.60±0.10[a]
Olive oil only	4.38±0.70[a]	2.95±0.56[a]	0.98±0.20[a]	0.66±0.13[a]
CCl_4 only	6.42±0.43[b]	5.20±1.17[b]	1.50±0.27[b]	1.22±0.35[b]
CCl_4 + 100mg/kg Extract	4.80±0.47[a]	3.65±0.38[a]	1.00±0.20[a]	0.75±0.07[a]
CCl_4 + 300mg/kg Extract	4.94±1.46[a]	3.47±0.93[a]	1.04±0.34[a]	0.73±0.22[a]
CCl_4 + 500mg/kg Extract	4.44±0.53[a]	2.90±0.18[a]	0.96±0.18[a]	0.64±0.20[a]
CCl_4 + 100mg/kg silymarin	4.32±0.19[a]	2.78±0.45[a]	0.96±0.15[a]	0.62±0.15[a]

n=5; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at $P<0.05$.

Table 4.8: Liver Function Parameters of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	DB (mg/dL)	TB (mg/dL)
Normal control	$39.40±2.97^{b}$	$59.00±5.83^{b}$	$154.80±17.23^{b}$	$8.20±0.72^{b}$	$13.50±1.70^{b}$
Olive oil only	$41.80±4.71^{b}$	$59.00±2.12^{b}$	$163.00±4.36^{b}$	$8.58±0.30^{b}$	$14.48±1.27^{b}$
CCl_4 only	$72.80±1.79^{ac}$	$118.60±10.26^{ac}$	$254.40±24.23^{ac}$	$30.60±1.57^{ac}$	$34.78±5.04^{ac}$
CCl_4 + 100mg/kg Extract	$55.40±9.45^{abc}$	$70.00±7.45^{ab}$	$213.40±18.97^{abc}$	$17.92±0.87^{abc}$	$20.42±1.32^{abc}$
CCl_4 + 300mg/kg Extract	$42.40±2.30^{b}$	$64.00±3.39^{b}$	$178.40±14.01^{b}$	$9.52±0.44^{b}$	$18.82±1.18^{ab}$
CCl_4 + 500mg/kg Extract	$42.60±2.41^{b}$	$60.60±4.42^{b}$	$166.00±4.42^{b}$	$9.18±0.35^{b}$	$18.02±1.78^{b}$
CCl_4 + 100mg/kg silymarin	$45.40±2.07^{b}$	$61.80±6.54^{b}$	$162.80±6.54^{b}$	$8.80±0.62^{b}$	$14.80±1.95^{b}$

n=5; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at P<0.05; a= significantly different from normal control group (P<0.05); b= significantly different from group induced with CCl_4 without extract or standard drug treatment (P<0.05); c= significantly different from group induced with CCl_4 but treated with standard drug (P<0.05).

Alanine Aminotransferase (ALP), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Direct Bilirubin (DB and Total Bilirubin (TB)

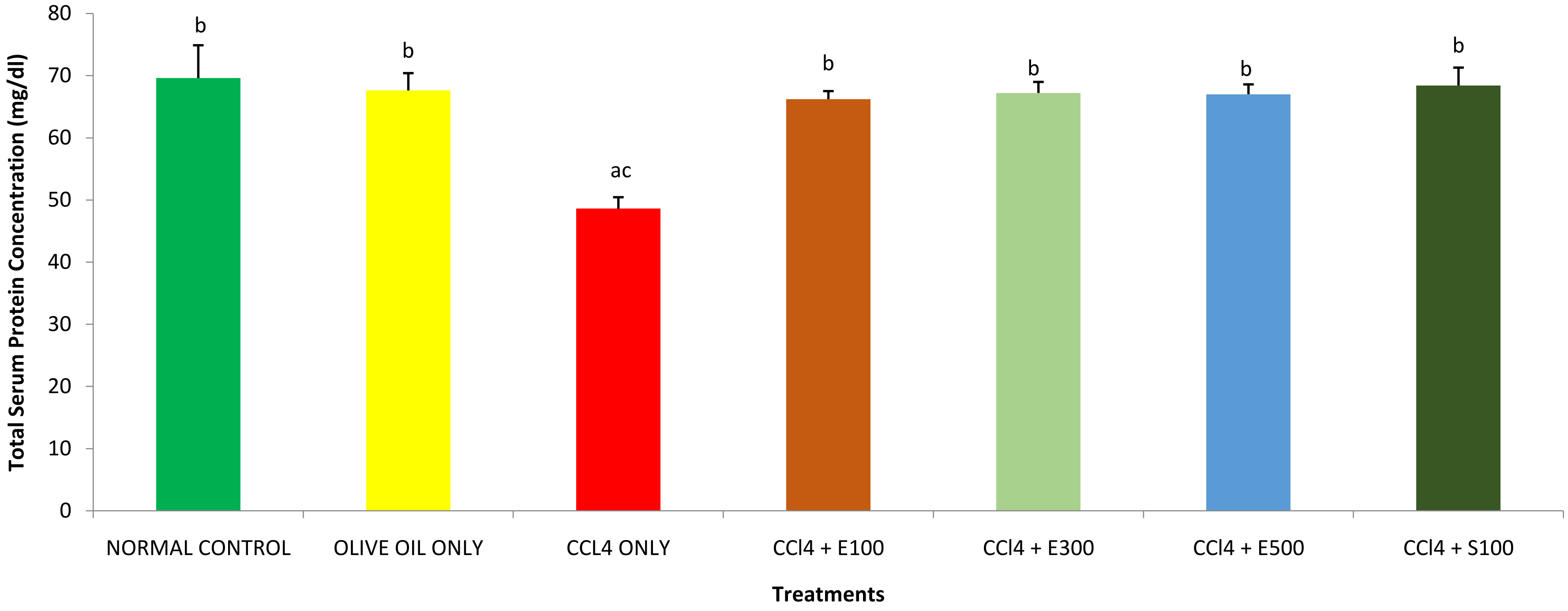


Figure 4.1: Serum Total Protein Concentration of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

n=5; Results are in mean±standard deviation; values with different superscript across the bars are significantly different at $P<0.05$; a= significantly different from normal control group ($P<0.05$); b= significantly different from group induced with CCl_4 without extract or standard drug treatment ($P<0.05$); c= significantly different from group induced with CCl_4 w but treated with standard drug ($P<0.05$).

CCl_4 + E100 = CCl_4 + 100mg/kg bw Extract; CCl_4 + E300 = CCl_4 + 300mg/kg bw Extract; CCl_4 + E500 = CCl_4 + 500mg/kg bw Extract; CCl_4 + S100 = CCl_4 + 100mg/kg silymarin

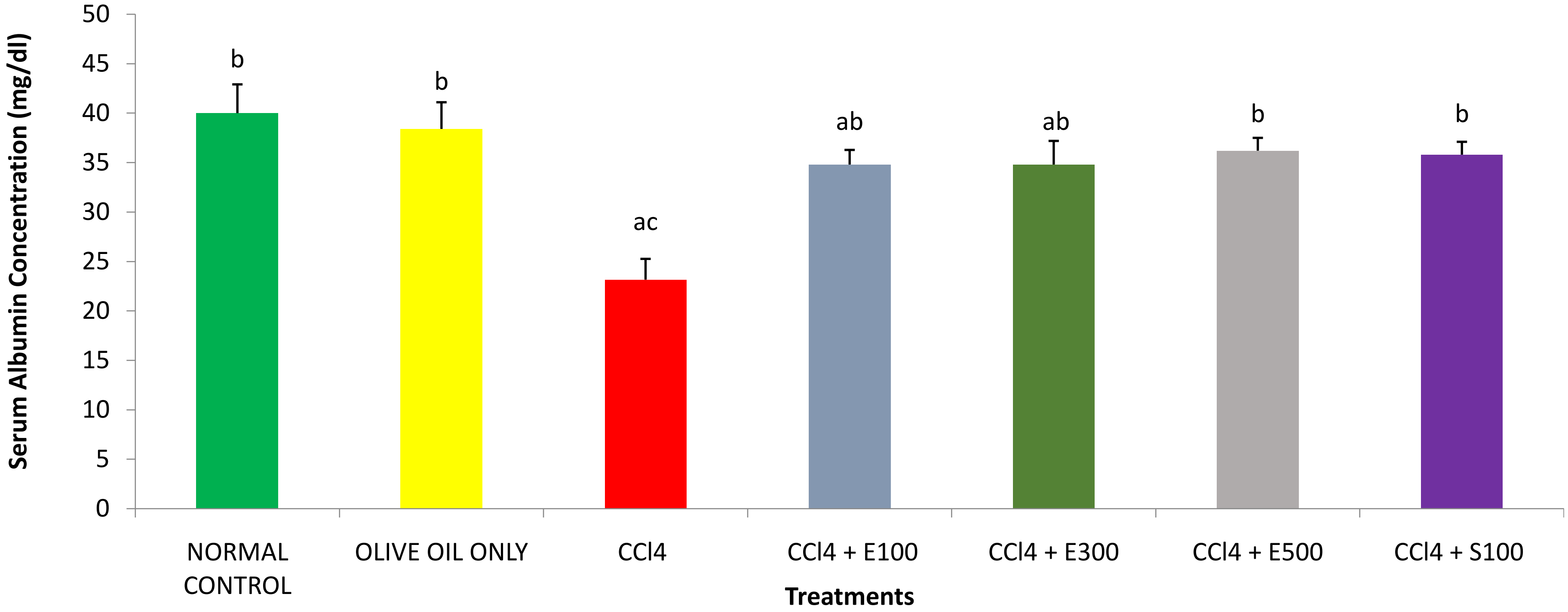


Figure 4.2: Serum Albumin Concentration of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fractio of Methanol Extract of *Ficus glumosa* Leaves

n=5; Results are in mean±standard deviation; values with different superscript across the bars are significantly different at $P<0.05$; a= significantly different from normal control group ($P<0.05$); b= significantly different from group induced with CCl_4 without extract or standard drug treatment ($P<0.05$); c= significantly different from group induced with CCl_4 w but treated with standard drug ($P<0.05$).

CCl_4 + E100 = CCl_4 + 100mg/kg bw Extract; CCl_4 + E300 = CCl_4 + 300mg/kg bw Extract; CCl_4 + E500 = CCl_4 + 500mg/kg bw Extract; CCl_4 + S100 = CCl_4 + 100mg/kg silymarin

4.2.3 Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves on Liver Antioxidant Status of Experimental Animals

The effect of CCl_4 pre-treatment followed by daily oral administration of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves for 21 days on antioxidant status in the liver of the experimental *albino* rats is presented in Table 4.9. Carbon tetrachloride significantly ($P<0.05$) increase the level of Malondialdehyde (MDA) with concomitant decrease in the activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in rats. However, treatment with 300 and 500 mg/kg body weight of extract and 100 mg/kg body weight of silymarin significantly reversed the status comparable to the normal control rats while 100 mg/kg body weight extract does not produce statistical significant ($P>0.05$) difference between the induced untreated control group.

4.2.4 Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves on Kidney Biomarkers of Experimental Animals

The result in Table 4.10 showed that, the concentrations of urea and creatinine in the serum of CCl_4-induced untreated *albino* rats were significantly ($P<0.05$) higher when compare to the induced treated groups. Similarly, the concentrations of electrolytes (sodium ion Na^+, potassium ion K^+, and chlorine ion Cl^-) in the serum of the experimental *albino* rats were significantly ($P<0.05$) raised in CCl_4-induced untreated group however, they were lowered by the extract and silymarin treatments comparable to the normal rats.

Table 4.9: Liver Antioxidant Status of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

Group	MDA (µM)	SOD (U/ml)	CAT (U/ml)	GPx (U/ml)
Normal control	1.16 ± 0.24^{b}	2.22 ± 0.11^{b}	53.80 ± 2.49^{b}	44.20 ± 2.59^{b}
Olive oil only	1.24 ± 0.17^{b}	2.10 ± 0.22^{b}	52.00 ± 1.58^{b}	40.60 ± 2.41^{b}
CCl_4 only	2.06 ± 0.17^{ac}	1.72 ± 0.08^{ac}	25.80 ± 3.77^{ac}	31.60 ± 2.07^{ac}
CCl_4 + 100mg/kg Extract	1.84 ± 0.11^{ac}	1.86 ± 0.15^{ac}	42.40 ± 2.88^{ac}	38.60 ± 2.61^{a}
CCl_4 + 300mg/kg Extract	1.40 ± 0.10^{b}	2.08 ± 0.13^{b}	43.40 ± 2.79^{ab}	39.40 ± 3.21^{b}
CCl_4 + 500mg/kg Extract	1.44 ± 0.11^{b}	2.02 ± 0.13^{b}	42.80 ± 2.77^{b}	39.80 ± 1.92^{b}
CCl_4 + 100mg/kg silymarin	1.46 ± 0.17^{b}	2.16 ± 0.11^{b}	44.40 ± 2.19^{b}	41.21 ± 2.39^{b}

n=5; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at $P<0.05$; a= significantly different from normal control group ($P<0.05$); b= significantly different from group induced with CCl_4 without extract or standard drug treatment ($P<0.05$); c= significantly different from group induced with CCl_4 but treated with standard drug ($P<0.05$).
MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

Table 4.10: Kidney Function Parameters of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

Group	Urea (mg/dl)	Creatinine (mg/dl)	Na+ (mmol/L)	K+ (mmol/L)	Cl- (mmol/L)
Normal control	3.50 ± 0.14^{b}	43.20 ± 5.45^{bc}	137.80 ± 2.59^{b}	3.98 ± 0.54^{b}	92.80 ± 1.92^{b}
Olive oil only	3.68 ± 0.66^{b}	42.00 ± 2.00^{bc}	132.20 ± 2.88^{b}	4.24 ± 0.36^{b}	97.40 ± 2.07^{b}
CCl_4 only	5.24 ± 0.52^{ac}	71.20 ± 4.66^{ac}	156.60 ± 4.51^{ac}	6.32 ± 0.41^{ac}	114.00 ± 7.91^{ac}
CCl_4 + 100mg/kg Extract	4.66 ± 0.17^{ab}	63.40 ± 2.70^{a}	139.80 ± 2.28^{b}	4.26 ± 0.18^{b}	100.00 ± 2.92^{b}
CCl_4 + 300mg/kg Extract	4.38 ± 0.24^{ab}	58.40 ± 6.47^{ab}	132.00 ± 2.12^{b}	4.28 ± 0.18^{b}	98.60 ± 3.05^{b}
CCl_4 + 500mg/kg Extract	4.12 ± 0.28^{b}	52.00 ± 6.41^{b}	138.80 ± 2.77^{b}	4.30 ± 0.29^{b}	99.40 ± 2.97^{b}
CCl_4 + 100mg/g silymarin	4.06 ± 0.38^{b}	56.20 ± 3.63^{ab}	137.00 ± 9.12^{b}	4.46 ± 0.23^{b}	97.80 ± 1.48^{b}

n=5; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at P<0.05; a= significantly different from normal control group (P<0.05); b= significantly different from group induced with CCl_4 without extract or standard drug treatment (P<0.05); c= significantly different from group induced with CCl_4 but treated with standard drug (P<0.05).
Sodium ion (Na^+), Potassium ion (K^+)and Chlorine (Cl^-)

4.2.5 Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves on Kidney Antioxidant Status of Experimental Animals

The effect of daily oral administration of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves for 21 days on the kidney antioxidant status of CCl_4-induced liver damaged rats is presented in Table 4.11. There was a significant ($P<0.05$) rise in the level of Malondialdehyde (MDA) with concomitant decrease activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the CCl_4-induced untreated rats as compared with the induced treated groups. However, there was no significant ($P>0.05$) difference in the levels of these parameters in the induced treated rats when compare to the normal control group as administration of *n*-butanol extract fraction (300 and 500 mg/kg body weight) and 100 mg/kg body weight silymarin were able to significantly (P<0.05) reverse the deplorable status of the antioxidant system as seen in the induced untreated rats.

4.3 Evaluation of Some Lipid Profile and Haematological Parameters of Experimental Animals

4.3.1 Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus Glumosa* Leaves on Serum Lipid Profile of Experimental Animals

In another evaluation, (Table 4.12), administration of *n*-butanol extract fraction and silymarin significantly ($P<0.05$) lowered the elevated levels of TCH, TAG and raised HDL level in CCl_4-induced treated rats compare to the induced untreated rats. However, there was no significant ($P>0.05$) difference in the levels of HDL, TCH and TAG of the induced treated rats as compared to the normal control group and the silymarin treated group.

Table 4.11: Kidney Antioxidant Status of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

Group	MDA (µM)	SOD (U/ml)	CAT (U/ml)	GPx (U/ml)
Normal control	0.34 ± 0.05^{b}	2.10 ± 0.07^{b}	41.60 ± 4.62^{b}	31.40 ± 3.05^{b}
Olive oil only	0.36 ± 0.05^{b}	2.04 ± 0.15^{b}	39.80 ± 3.96^{b}	28.60 ± 6.22^{b}
CCl_4 only	0.84 ± 0.05^{ac}	1.68 ± 0.04^{ac}	17.00 ± 2.00^{ac}	15.20 ± 0.04^{ac}
CCl_4 + 100mg/kg Extract	0.78 ± 0.05^{ac}	1.82 ± 0.08^{ac}	32.40 ± 2.88^{ab}	26.60 ± 4.93^{b}
CCl_4 + 300mg/kg Extract	0.44 ± 0.08^{b}	2.02 ± 0.12^{b}	33.00 ± 0.71^{b}	27.40 ± 3.21^{b}
CCl_4 + 500mg/k Extract	0.36 ± 0.05^{b}	2.00 ± 0.10^{b}	40.60 ± 4.39^{b}	30.40 ± 3.21^{b}
CCl_4 + 100mg/kg silymarin	0.36 ± 0.06^{b}	2.10 ± 0.07^{b}	42.40 ± 3.78^{b}	27.05 ± 6.40^{b}

n=5; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at $P<0.05$; a= significantly different from normal control group ($P<0.05$); b= significantly different from group induced with CCl_4 without extract or standard drug treatment ($P<0.05$); c= significantly different from group induced with CCl_4 but treated with standard drug ($P<0.05$).
MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

Table 4.12: Serum Lipid Profile of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

Group	TCH (mmol/L)	TAG (mmol/L)	HDLc (mg/dL)
Normal control	$1.84±0.27^{b}$	$0.54±0.11^{b}$	$1.44±0.43^{b}$
Olive oil only	$2.26±0.18^{b}$	$0.62±0.08^{b}$	$1.04±0.43^{b}$
CCl_4 only	$9.04±0.83^{ac}$	$1.26±0.11^{ac}$	$0.42±0.08^{a}$
CCl_4 + 100mg/kg Extract	$2.52±0.28^{b}$	$0.88±0.19^{ab}$	$0.64±0.17^{a}$
CCl_4 + 300mg/kg Extract	$2.30±0.16^{b}$	$0.80±0.07^{b}$	0.92±0.13*
CCl_4 + 500mg/kg Extract	$2.18±0.20^{b}$	$0.78±0.19^{b}$	$1.14±0.11^{b}$
CCl_4 + 100mg/kg silymarin	$2.28±0.23^{b}$	$0.78±0.15^{b}$	0.94±0.18*

n=5; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at P<0.05; a= significantly different from normal control group (P<0.05); b= significantly different from group induced with CCl_4 without extract or standard drug treatment (P<0.05); c= significantly different from group induced with CCl_4 but treated with standard drug (P<0.05); *= not significantly different from any of the three control references.
Total Cholesterol (TCH), Triacylglycerol (TAG) and High Density Lipoprotein (HDL)

4.3.2 Effect of *n*-Butanol Fraction of Methanol Extract of *Ficus Gglumosa* Leaves on some Haematological Parameters of Experimental Animals

The effect of daily oral administration of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves for 21 days on some haematological parameters (packed cell volume PCV haemoglobin HB and white blood cell WBC) of CCl_4-induced liver damaged rats is shown on Table 4.13. There was a significant (*P*<0.05) decrease in the level of packed cell volume and haemoglobin in the CCl_4-induced untreated group as compared to the induced rats that were treated with *n*-butanol extract fraction and silymarin. Meanwhile, there was no significant (*P*>0.05) difference in the levels of PCV and HB of the induced treated groups when compared with the normal rats. It also showed a significant increase (P<0.05) in WBC in the CCl_4-induced untreated group as compared to the normal and CCl_4-induced treated groups.

Table 4.13: Some Haematological Parameters of CCl_4-induced Liver Damaged Albino Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves

Group	PCV (%)	Hb (g/dL)	WBC (10^3/L)
Normal control	40.40 ± 2.41^{b}	11.42 ± 0.24^{b}	08.22 ± 1.00^{b}
Olive oil only	41.00 ± 4.30^{b}	11.16 ± 1.72^{b}	07.00 ± 0.43^{b}
CCL4 only	20.20 ± 1.92^{ac}	05.42 ± 0.41^{ac}	13.68 ± 1.87^{ac}
CCL4 + 100mg/kg Extract	26.20 ± 6.01^{ac}	08.98 ± 1.06^{b}	08.90 ± 0.54^{b}
CCL4 + 300mg/kg Extract	34.20 ± 1.30^{ab}	09.00 ± 2.09^{b}	08.98 ± 1.77^{b}
CCL4 + 500mg/kg Extract	37.40 ± 1.14^{b}	09.36 ± 0.93^{b}	08.64 ± 1.39^{b}
CCL4 + 100mg/kg silymarin	39.20 ± 2.45^{b}	09.38 ± 1.32^{b}	07.86 ± 1.07^{b}

n=5; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at $P<0.05$; a= significantly different from normal control group ($P<0.05$); b= significantly different from group induced with CCl_4 without extract or standard drug treatment ($P<0.05$); c= significantly different from group induced with CCl_4 but treated with standard drug ($P<0.05$).

Packed Cell Volume (PCV), Haemoglobin (Hb) and White Blood Cell (WBC)

CHAPTER FIVE

5.0 DISCUSSION

Medicinal plants have been appreciated for a very long time for their numerous pharmacological effects which are attributed to the presence of secondary metabolites like alkaloids, flavonoids, phenols, glycosides tannins and saponnins (Fatma *et al.,* 2013). Some of these plants have been evaluated for antioxidant property which is essential to limit the risk and progression of certain acute and chronic diseases (Ali *et al.,* 2008).

From this study, the presence of saponins, tannins, alkaloids and flavonoids in the crude methanol extract of *Ficus glumosa* leaves correlates with the earlier report by Tanko *et al.* (2012) that showed the presence of these compounds in methanol leaves extract of this plant. These results may indicate possible cellular protective effect of *Ficus glumosa* against oxidative damage probably owe to the antioxidative properties of these compounds (Jayathilakan *et al*., 2007). Quantitatively, flavonoids, phenols and ascorbic acid were found in varying concentrations in the *n*-butanol, ethylacetate, *n*-hexane and aqueous fraction of crude methanol leaves extract of *Ficus glumosa*. This variation may be however, attributed to the difference in polarity of the solvents and molecular size of compounds present in the plant extracts (Jongkwon *et al.,* 2014; Ali *et al.*, 2011).

Plant phenolic acids, flavonoids and ascorbic acid constitute major groups of phytochemicals acting as primary *in vitro* antioxidants or free radical scavengers (El-Sayed *et al*., 2012). Therefore, it was reasonable to determine their concentrations in the various fractions with the aim of utilising the fraction with the highest concentration of these *in vitro* antioxidants. Free radicals scavenging potentials of *n*-butanol, aqueous, ethylacetate and *n*-hexane fractions may depend on phenolic acids, flavonoids and ascorbic acid unique structure, number and position of the hydroxyl groups as reported by Pazos *et al*. (2005). The low IC_{50}value (0.42±0.04mg/ml)of *n*-butanol fraction suggests it is a better free radical scavenger compared to ethylacetate, *n*-hexane and aqueous fractions. This result is in conformity with Jamuna *et al.* (2014), which showed an inverse relationship between IC_{50} and free radical scavenging activity of *Hypochaeris radicata*.

The high LD_{50} (>5000mg/kg) of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves may be due to the presence of useful phytochemical constituents that serve as protective mechanism to the cells rather than poison.

Percentage change in organ weights after CCl_4 intoxication has been used as a valuable index of organ damage and possible biochemical changes within biological system (Akram *et al.*, 2012). The significant increase (P<0.05) in percentage relative liver and kidney weights seen in CCl_4-induced rats was in agreement with the report of Akram *et al.* (2012) and may be possibly considered to be as a result of direct toxicity and/or indirect toxicity of $CCl_4$to liver and kidney causing hypertrophy of the cells of these organs by possibly increasing their fluid retention and cellular proliferations (Li *et al*., 2011). In contrast, all the induced but treated groups experienced a significant reduction (P<0.05) in organ weights, suggesting the possible ameliorative effects of *n*-butanol fraction ofmethanol extract of *Ficus glumosa* leaves against CCl_4-induced liver and kidney injuries.

Administration of carbon tetrachloride (CCl_4) caused a significant elevation (P<0.05) of liver marker enzymes levels and a concomitant decrease in the level of serum total protein and albumin in rats. These observations were in agreement with previous reports that the activity levels of serum ALT, ALP and AST were significantly elevated with a corresponding significant (P<0.05) decrease in serum protein and albumin levels in rats after CCl_4 administration (Akram *et al*., 2012; Ama *et al*., 2013; Muhammad *et al.*, 2013; Abiodun *et* *al.*,2014). The obtained resultscould probably indicate a severe liver damage caused by CCl_4 which may had led to an impaired protein turnover from the liver as well as leakage of these liver enzymes from the damaged liver (Atawodi *et al.,* 2013; Jude *et al*., 2016). In the other hand, the significant elevation ($P<0.05$) of total and direct bilirubin levels in the CCl_4-induced rats correlates with the findings of Babalola *et al*. (2011) and Aluko *et al.* (2013) where bilirubin concentration was elevated as a result of hepatic injury caused by paracetamol. Meanwhile, this elevation may be as a result of haemolytic anaemia associated with oxidative damage to red blood cells or internal haemorrhage thus, leading to elevated bilirubin levels (prehepatic jaundice) since bilirubin is an intermediate metabolic product of haeme group from haemoglobin or myoglobin breakdown in the liver (Reham *et al*., 2009). Possibly, this elevated bilirubin levels may also be associated with reduced hepatocyte uptake of bilirubin, impaired conjugation of bilirubin (Dominic *et al*., 2012; Khan *et al*., 2012). As well, it may be attributed to the possible obstruction in the flow of bile within the liver (hepatic jaundice) or in the bile duct (post jaundice) due to distortion of membrane transporters such as ABC, MRP and OATP at the canalicular and sinusoidal membranes (Evan and Milan, 2013; Battu *et al.*, 2012). However, administration of the extract fraction and sylimarin significantly ($P<0.05$) reversed the impact of CCl_4 on these liver marker enzymes activities, bilirubin, serum protein and albumin levels to a near normal as seen in the work of Babalola *et al*. (2011), Aluko *et al.* (2013) and Jude *et al*. (2016) where leaves extracts of *Hyptis suaveolens*, *Ocimum americanum* and stem-bark extract of *Mammea Africana* equally exhibited similar actions respectively. These results may possibly indicate the ameliorative potency of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves against hepatoxicity induced by CCl_4. The reversal of these serum liver marker enzymes in CCl_4-induced treated groups near normalcy by the *n*-butanol fraction observed in this study may be due to the prevention of the leakage of these intracellular enzymes as a result of the presence of phenols, flavonoids and ascorbic acid in the *n*-butanol fraction owe to their membrane stabilizing activity which may be attributed to their ability to mop up free radicals generated by CCl_4that attack cell membranes (Chavan *et al*., 2012) which is in agreement with the commonly accepted view that serum levels of transaminases, bilirubin and serum proteins returned back to normalcy with the healing of hepatic parenchyma cells which lead to the regeneration of hepatocytes (Mariam *et al.*, 2015; Chavan *et al*., 2012).

The level of thiobarbituric acid reactive substances such as malondialdehyde and the activity of endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase is a sensitive index in free radical induced hepatocellular damage (Mohajeri *et al*., 2011). The significant increase ($P<0.05$) in the levels of Malondialdehyde (MDA) in the liver and kidney tissues of CCl_4-induced rats may be as a result of an enhanced membrane lipid peroxidation by free radicals generated by CCl_4 and the failure of the antioxidant defence mechanisms in the system to reduce the excessive formation or detrimental effects of such free radicals probably because the endogenous antioxidants have been overwhelmed (Kim *et al*., 2010). In contrast, the significant decreased ($P<0.05$) activities of SOD, GPx and CAT in the liver and kidney tissues of CCl_4-induced rats may be due to high concentration of free radicals generated by CCl_4which may have led to inactivation (adaptive response) or probably inhibition of the synthetic pathways of these endogenous antioxidant enzymes thereby resulting in their low turnover (Showkat *et al*., 2010). However, treatments with *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves and silymarin appear to restore the levels of SOD, GPx and CAT activities to near normal as well as reduced the MDA level which was in conformity with the report of Momoh, *et al.* (2015) where administration of *Vernonia amygdalina* aqueous leaves extract equally exhibited similar actions against liver damage induced by acetaminophen. The effects of the *n*-butanol fraction were comparable to the standard drug (Silymarin) which equally increased the activity of the endogenous antioxidant enzymes. Thus, this result could be attributed to the free radical scavenging activity of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves demonstrated earlier due to the presence of antioxidant phytochemicals such as phenolic acids, flavonoids and ascorbic acid which may have exerted beneficial action against pathophysiological alterations caused by the presence of superoxide and hydroxide free radicals as well as hydrogen peroxide thereby restoring the antioxidant status in the cells (Etim *et al.*, 2008).

Similarly, administration of CCl_4 caused nephrotoxicity as indicated by significant elevated ($P<0.05$) urea and creatinine levels as well as electrolytes (Na^+ K^+ and Cl^-) concentrations in the serum of the experimental animals. These results are in agreement with earlier findings by Venkatanarayana *et al*. (2012) and Yacout *et al*. (2012). From this study, it is evident that significant elevation in serum urea, creatinine and electrolytes levels can be attributed to damaged nephron structural integrity (Khan and Siddique, 2012). However, the significant reduction ($P<0.05$) in urea, creatinine and electrolytes levels in the CCl_4-induced but treated groups can be ascribed to the ameliorative potency of the *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves on the glomerular and tubular cells which may have improved renal function in the diseased kidney.

The lowered levels of high density lipoprotein (HDLc) and increased total cholesterol (TC) and triacylglyceride (TAG) in the CCl_4-induced untreated group were in agreement with earlier findings by Adejo *et al.* (2014) where CCl_4 was able to significantly ($P<0.05$) caused similar effects in rats. These observations could be an indication of metabolic distortion in the liver due to the severity of hepatic injury inflicted by CCl_4 (Adejo *et al.*, 2014). However, treatments with the *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves and silymarin reversed the status to a near normal. This ameliorative effect may be owed to the ability of the bioactive compounds in the extract to increase lipase activity which hydrolyses

TAG (Adejo *et al.,* 2014) or possibly may have acted as inhibitor to hydroxyl-methyl-glutaryl-CoA reductase, a key enzyme in the *de novo* biosynthetic pathway of cholesterol (Gebhardt and Beck, 1996), otherwise chelated the by-products of CCl_4 metabolism thereby stabilizing the liver regulatory roles (Dawood *et al*., 2014)

Furthermore, CCl_4 reactive species [trichloromethyl (CCl_3^o) and trichloromethylperoxy (CCl_3OO^o)] might have possibly caused the significant ($P<0.05$) transient decrease in the Hb concentration and PCV level due to haemolytic anaemia caused by oxidation of sulphydryl groups of the erythrocyte membrane in addition to disturbed hematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and/or their enhanced removal from circulation (Khalid *et al.,* 2013; Maduka *et al.,* 2014; Mariam *et al.,* 2015). On the other hand, the CCl_4 treatment significantly ($P<0.05$) increased WBCs count which may be attributed to lymphocyte infiltration of poisoned cells, a clear case of immune response to a chemical antigen by the body defensive mechanism of immune system (Saba *et al.,* 2010).However, treatment with *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves showed significant ($P<0.05$) reversal effects of these indices comparable with silymarin and normal control rats. The consequent reduction in red blood cells haemolysis and enhanced haematopoiesis with the decrease in the WBCs count may be ascribed to the stabilization of the free radicals by some antioxidants present in the *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves, an effect that was in agreement with the findings of Saba *et al.* (2010) where aqueous extract of *Cnidoscolus aconitifolius* leaves produced similar action against CCl_4-induced hepatotoxicity and haemotoxicity in rats.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The findings from this study can be summarised as follows:

i. Qualitative phytochemical screening of the crude methanol extract of *Ficus glumosa* leaves showed the presence of flavonoids, saponnins, tannins, and alkaloids whereas quantitatively, phenolics, flavonoids and ascorbic acid were found in varying amounts in the different fractions of the methanol extract of *Ficus glumosa* leaves.

ii. The free radical scavenging analysis of the solvent fractions of methanol extract of *Ficus glumosa* leaves demonstrated that *n*-butanol is the most active fraction with IC_{50} value of 0.41±0.07 mg/ml.

iii. The *n*-butanol fraction exhibited anti-hepatotoxic effect as evident by the significant ($P<0.05$) reduction in the levels of serum liver marker enzymes, bilirubin as well as increased serum proteins and reversal of antioxidant status in the liver of the oxidative stressed animals especially at 300 and 500 mg/kg per body weight.

iv. Anti-nephrotoxic effect of *n*-butanol fraction was evident by the significant ($P<0.05$) reduction in the levels of creatinine, urea and electrolytes as well as the reversal of antioxidant status in the kidneys of the experimental animals especially at 300 and 500 mg/kg per body weight.

v. There was also hypolipidaemic effect observed from the significant ($P<0.05$) reduction of total cholesterol with corresponding increased HDL. Similarly, the *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves causeda gradual increase in PCV and Hb levels with corresponding decrease in WBC in the oxidative stressed rats.

6.2 Conclusion

The results from this study demonstrate that the *n*-butanol fraction of methanol extract of *Ficus glumosa*leaves is a good source of antioxidants which may have contributed to its significant ameliorative effects on the damaged biochemicalparameters of *albino* rats induced with carbon tetrachloride.

6.3 Recommendations

Due to the rich antioxidant properties of*n*-butanol fraction of this plant, it may be very useful in the treatment of oxidative stress related diseases if properly refined.

Furthermore, elaborate experimental design could be plan to evaluate the mechanism of anti-hepatotoxicity and anti-nephrotoxicity activity of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves in future.

In addition, studies should be carried out on the bioactivity-guided fractionation, isolation and identification of the bioactive constituents of the *n*-butanol fraction which is responsible for the observed pharmacological activities.

REFERENCES

Abiodun, H. A., Fuhang, S., Xue, T., Liu, Q., Huan, D., Pei, H., Ji Yu, Z. and Li Xin, Z. (2014). *Citrilus lamatus* extract reverses oxidative and haematological dysfunctionin carbon tetrachloride induced liver damage in rats. *International Journal of Pharmacology,* 10(4): 218-224.

Adaeze, B.E., Yvonne, O.C., Sylvester, I.O. and Oghenakhogie, I.M. (2015). *Phyllanthus amarus:* A hepatoprotective agent in acetaminophen-induced liver toxicity in adult wistar rats. *Sikkim Manipal University Medical Journal*, 2(1): 150-165.

Adejo, G.O., Atawodi, S.E., Ameh, D.A and Ibrahim, S. (2014). Anti-peroxidative, protective and ameliorative properties of methanol extract of all parts of *Morinda lucida* (Benth) in CCl_4-induced liver injury. *Natural Product Chemistry and Research*, 1:1-7.

Aebi, H.E. and Bergmeyer, H.U. (1983). Methods in enzymatic analysis. *New York Academic press Inc.,* 3: 276-286.

Aigbokhan, E.I. (2014). Annotated checklist of vascular plants of southern Nigeria: a quick reference guide to vascular plants of southern Nigeria: a systematic approach, University of Benin Press, Benin City, p89.

Aja, P.M., Ezeudeh, N.L., Umahi, B.G., Ugwu-okechukwu, P.C., Enenchi, O.C., Nweke, O.L. and Patience N.O. (2016). Hepatoprotective effect of ethanol extract of *Pterocarpus santalinoides* leaf on carbon tetrachloride (CCl_4) induced *albino* rats. *Caribbean Journal of Science and Technology,* 4: 882-895.

Akharaiyi, F.C., Boboye, B.E. and Adetuyi, F.C. (2012). Hepatoprotective effect of ethanol leaf extract of *cnestis ferruginea* on swiss *albino* mice induced with paracetamol. *International Research Journal of Pharmaceuticals,* 2(4): 120-126.

Akram, E., Pejman, M., Masoud, E.T., Ali, H. R. and Shahabaldin, S. (2010). Hepatoprotective effect of pantothenic acids on carbon tetrachloride-induced toxicity in rats. *Experimental Clinical Sciences Journal*, 11: 748-759.

Alexander, R.R. and Grifiths, J.M. (1993a and 1993b). Haemoglobin determination by the cyanmethaemoglobin method. In Alexander R. R. and J. M. Grifiths (Eds.), New York: John Wiley and sons. Basic biochemical method, p188-189.

Alfred, O. (2014). The behaviour of moraceae *Ficus glumosa* delile as a corrosive inhibitor for zinc H_2SO_4. *Chemical Science Review and Letters,* 3(11S): 49-55.

Ali, G.,, Hawa, Z.E.J., and Asmah R. (2011). Effects of solvent type on phenolics and flavonoids contents and antioxidant activity in two varieties of young ginger (*Zinger officinale* Roscoe) extracts. *Journal of Medicinal Plants Research*, 5(7): 1147-1154).

Ali, S.S., Kasoji, N., Luthra, A., Sharanabasava, H. and Sahu, A. (2008). Indian medicinal herbs as source of antioxidants. *Food Research International,* 4(1): 1-5.

Aluko, B.T., Oloyede, O.L. and Afolayan, A.J. (2013). Hepatoprotective activity of *Ocimum americanum* leaves against paracetamol-induced liver damage in rats. *American Journal of Life Sciences,* 1(2): 37-42.

Ama, U.I., Emmanuel, I.U., Christ, E. and Okechukwu, U. (2013). Cadmium-induced toxicity and the hepatoprotective potentials of aqueous extract *Jessiaea nervosa* leaf. *Advance Pharmaceutical Bulletin*, 3(2): 309-313.

Amador, E and Wacker, W (1962). Analytical methods for quantitative determination of liver marker enzymes, *ClinicalChemistry*, 1962: 8:343.

Ameh, P.O. (2013). Physiochemical properties and rheological behaviour of *ficus glumosa* gume in aqueous solution. *African Journal of Pure and Applied Chemistry,* 7(1): 35-43.

Arhoghro, E.M., Ike, C.H., Eboh, A.S. and Angalabiri, O.B. (2015). Liver function of wistar rats fed with combined ethanolic leaf extracts of *Alcornea cordifolia* and *Costus afer* in paracetamol-induced toxicity. *World Journal of Pharmaceutical Research,* 4(5): 01-12.

Atawodi, S.E., Ojochenemi, E.Y., Mubarak, L.L. and Dorothy, U.I. (2014). Effects of methanolic extract *Tetrapleura tetraptera* (Schum and Thonn) taub leaves on hyperglycaemic indices of diabetic complications in alloxan-induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine,* 4(4): 272-278.

Atawodi, S.E., Yakubu, O.E. and Umar, I.A. (2013).antioxidant and hepatoprotective effects of *Parinari curatellifolia* root*. International Journal of Agriculture and Biology,* 15: 523-528

Azubike, N.C., Achukwu, P.U., Okuosa, C.N. and Oduah E. (2015). Evaluation of hepatoprotective activity of *Colocasia esculenta* (L. schott) leaves on thioacetamide-induced hepatotoxicity in rats. *Pakistan Journal of Pharmaceutical Sciences,* 2(6): 2237-2241.

Babalola, O.O., Ojo, O.E. and Oloyede, F.A. (2011). Hepatoprotective activity of aqueous extract of the leaves of *Hyptis suaveolens* (I.) poit on acetaminophen induced hepatotoxicity in rabbit. *Research Journal of Chemical Sciences,* 1(7): 85-88.

Barros, L., Joao F. M., Queiros, B., Ferreira, I.C. and Baptista P. (2007). Total phenol, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushroom and their antioxidant acitivities. *Food Chemistry,* 413-419.

Battu, G., Rao, Y., Venkateswara, D.V.S. and Praneeth (2012). Antihepatotoxic effect of Elephantopus scaber L. on carbon tetrachloride-induced hepatotoxicity in rats. *Recent Research in Science and Technology,* 4(4): 21-24.

Bertels, H. and Bohmer, M. (1973). Serum Creatinine determination without protein precipitation, *Clinica chimica Acta*, 37: 193.

Bhalodia, N., Nariya, P., Acharya, R. and Shukla, V. (2011). Evaluation of in vitro antioxidant activity of flowers of *Cassia fistula* Lin. *International Journal of PharmTech Research,* 3(1): 589-599.

Biff, F. P. (2015). Regulation of potassium homeostasis. *Clinical Journal of American Society of Nephrology*, 10(6): 1050-1060.

Buraimoh, A.A., Bako, I.G., and Ibrahim, F.B. (2011). Hepatoprotective effect of ethanolic leave extract of *Moringa oleifera* on the histology of paracetamol induced liver damage in wistar rats. *International Journal of Animal and Veterinary Advance,* 3: 10-13.

Carmen, M., Anthonio, J. L., Jose, M., Calderon, M., Estefania, B. and Miguel, L. (2012). Proxidant natural product as anticancer. *Current Drug Target,* 13(8): 1006-1028.

Chavan, S., D., Patil, S. B. and Naikwade, N.S. (2012). Biochemical and Histopathological Studies of *Butea Monosperma* (Lam) Taub Leaves on Paracetamol-Induced Hepatotoxicity in Albino Rats. *Journal of Pharmacy Research,* 5(8):4006-4008.

Cheesbrough, M. (2000). District laboratory practice in tropical countries. (part 2). Cambridge. Cambridge University Press. Pp. 297-298.

Craft, B.D., Kerrihard, A.L., Amarowicz, R. and Peggi, R.B. (2012). Phenol-based antioxidants and the invitro methods used for their assessment. *Compress Review Food Science,* 11: 148-173.

David, M. S., Jeff, S.C., Nigel, B., David, W. J and Glenda C. G. (2012). Oxidative stress, antioxidant therapies and chronic kidney disease. *Nephrology,* 17: 311-321.

David, W. I., William E. M. and Jeff, M. S. (2015). Urea and ammonia metabolism and the control renal nitrogen excretion. *Clinical Journal of American Society of Nephrology*, 10(8): 1444-1458.

Dawood, A., Muhammad, F., Sheeraz, M. A., Habiba, N., Hina, G. and Saira, A. (2014). Protective action of *Taraxcum officinale* on CCL4-induced hepatotoxicity in rats. *African Journal of Pharmacy and Pharmacology*, 8(30): 775-780.

Dominic, A. A., Parkavi, C., Murugaiah, K. and Dhanaraj, T.S. (2012).Hypolipidemic Activity of *Cyperous rotundus* on CCl_4-Induced Dyslipidemia in Rats. *Asian Jounal of Pharmaceutical Technology,* 2(2):51-53.

Doumas, B.T., Watson, W.A. and Biggs, H.G. (1971). Albumin standards and the measurement,*Clinica Chimica Acta,* 31: 87-96.

Ebong, P.E., Igile, G.O., Mgbeje, B.A., Iwara, I.A., Odong, A.E., Onufiok, U.L. and Oso, E.A. (2014). Hypoglycaemic, hepatoprotective and neuroprotective effects of methanolic leave extract of *Heinsia crinite (Rubiaceae)* in alloxan-induced diabetic *albino* wistar rats. *Journal of Pharmacy,* 4(1): 37-43.

Edgar, J. D., Elias, B. and Adnan, B. (2002). Biotechnology and the developing world. *Electronic Journal of Biotechnology*, 5(1): 0717-3458.

Effiong, G.S., Udoh, I.E., Udoh, N.M., Asuquo, E.N., Wilson, L.A, Ntukidem, I.U. and Nwoke, I.B. (2013). Assessment of hepatopotective activity of *Nauclea latifolia* leaf extract against acetaminophen-induced hepatotoxicity in rats. *International Research Journal of Plant Science,* 4(2): 53-63.

El-Sayed, S., Abdel-Hameed, S.A., Bazaid and Mohamed M. S. (2012). Total Phenolics and Antioxidant Activity of Defatted Fresh Taif Rose, Saudi Arabia. *British Journal of Pharmaceutical Research*, 2(3): 129-140.

Elujoba, A.A., Odeleye, O.A. and Ogunyemi, C.A. (2005). Traditional medicine development for medical and dental primary healthcare delivery system in Africa. *African Journal of Traditional Complementary and Alternative Medicine, 2(1): 46-61.*

Enogieru, A.B., Charles, Y.O., Omoruyi, S.I., Momodu, O.L. and Ezeuko, V.C. (2015). Stem-bark of Ficus exasperate protects the liver against paracetamol-induced toxicity in wistar rats. *Journal of Applied Sciences and Environmental Management,* 19(1): 155-159.

Erik, H., Lewis, E. D., Horst, G., William, H. D. and Dirk, W. (2014). Osmoregulation and excretion. *American Physiology Society*, 4: 405-573.

Etim, O.E., Akpan, E.J. and Usoh, I.F., (2008). Hepatotoxicity of carbon tetrachloride: protective effect of *Gongronema latifolium*. *Pakistan Journal of Pharmaceutical Sciences,* 21 (3):269–274.

Evan, S. and Milan, J. (2013). New insight in bilirubin metabolism and their clinical implications. *World Journal of Gastroenterology*,19(38): 6398–6407.

Ezeonwu, V.U. and Dahiru, D. (2013). Protective effect of *Ocimum gratissimum* and *Gongronema latifolium* aqueous leaf extracts on acetaminophen-induced hepato-nephrotoxicity in rats. *American Journal of Biochemistry,* 3(1): 18-23.

Fabricant, D.S. and Farnsworth, N.R. (2001). The values of plants used in traditional medicine for drug discovery. *Environmental Health Perspective*, 109(1); 69-75.

Fatma, A., Sokindra, K. and Shah, A.K. (2013). Estimation of total phenolic content, *in vitro* antioxidant and anti-inflammatory activity of flowers of *moringa oleifera*. *Asian Pacific Journal of Tropical Biomedicine*, 3(8): 623-627.

Fawcett, J.K. and Scout, J.E. (1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*, 13: 156.

Fidele, N., Djedouboum, A., Blaise, K., Paulin, N., Adjia, H. and Theophile, D. (2014b). Acute and sub-chronic oral toxicity of aqueous extract leaves of *ficus glumosa* del.(*moraceae*) in rodents. *Journal of Intercultural Ethnopharmacology,* 3(4): 206-213

Fidele, N., Djedouboum, A., Blaise, K., Paulin, N., Christian, B., Maguirgue, K., Emmanuel, T., and Theophile, D. (2014a). Diuretic activity of the aqueous extract leaves of *ficus glumosa* del.(*moraceae*) in rats. *The Scientific World journal*, 2014(693803): 2-10.

Fine, J. (1935). Quantitative determination of serum proteins by colorimetric method,*Biochemistry Journal*, 29:799.

Fraga, C.G., Leibovitz, B.E. and Toppel, A.L. (1988). Lipid peroxidation measured as TBARS in tissue slices: Characterization and comparison with homogenates and microsomes. *Free Radical Biology in Medicine*, 4: 155-161.

Gazak, R., Watterova, D. and Kren, V. (2007). Silybin and silymarin- new and emerging applications in medicine. *Current Medicinal Chemistry*, 14: 315-338.

Gebhardt, R. and Beck. H. (1996). Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte culture. *Lipids*, 31: 1269-1276.

Gholamreza, K., Maryam, V., Parisa, L., Marziyeh, R. and Mohammed, M. (2011). “Silymarin”, a promising pharmacological agent for treatment of diseases. *Iranian Journal of Basic Medical Sciences*, 14(4): 308-317.

Ghosh, A., Ghosh, T. and Jain, S. (2010). Silymarin- a review on the pharmacodynamics and bioavailability enhancement approaches. *Journal of Pharmaceutical Science and Technology*, 2(10): 348-355.

Halliwell, B. (2010). Free Radical and antioxidant- Quo Vadis?. *Trends in Pharmacology Science*, 32: 125-130.

Hamouda, H.E., Zakaria, S.S., Ismail, S.A., Khedr, M.A. and Mayah, W.W. (2011). P53 antibodies, metallothioneins and oxidative stress markers in chronic ulcerative colitis with dysplasia. *World Journal of Gastroenterology*, 2011, 17: 2417-2423.

Hassan, S.W., Salawu, K., Ladan, M.J., Hassan, L.G., Umar, R.A. and Fatihu, M.Y. (2010). Hepatoprotective, antioxidant and phytochemical properties of leaf extracts of *Newbouldia laevis.International Journal of PharmTech Research*, 2(1): 573-584.

Hassan, S.W., Tillo, M.K., Lawal, M., Umar, R.A, Ndakotsu, M.A., Farouk, U.Z. and Agaie, B.M. (2015). Hepatoprotective action of stem-bark extracts of *Newbouldia laevis* in rats treated with carbon tetrachloride. *Journal of Global Biosciences*, 4(3): 1627-1646.

Haussament, T.U. (1977). Quantitative determination of serum alkaline phosphatase,*Clinica Chimica Acta*., 35:271-273.

Heard, K.J. (2008). Acetacystein for acetaminophen poisoning. *The New England Journal of Medicine,* 359(3): 285-292.

Hikino, H., Kiso, Y. and Wagner, H. (2000). Antihepatotoxic actions of flavonolignans from Silybum marianum fruits. *Planta Medicine*, 50 (3):248-250.

Innocent, A.E., Koofreh, G.D., Blessing, C.A., Chrotopher, C.M. and Wonderful, U.U. (2014). Ethanol extract of *Emilia sonchifolia* possess erythropoetic and hepatoprotect effect in mice infested with *Plasmodium berghei berghei.Macedonian Journal of Medical Sciences,* 2(1): 11-17.

Ivana, C., Catia, C., Francesco, P., Andriano, M. and Anthonio, S. (2010). Prodrug approach for increasing cellular gluttathone levels. *Molecules,* 15: 1242-1264.

Iwuanyanwu, K.C., Wegwu, M.O. and Okiyi, J.K. (2010). Hepatoprotective effect of African locust bean (*Parkia clappertoniana*) and negro pepper (*Xylopia aethiopica*) in CCl_4-induced liver damage in wistar *albino* rats. *Experimental and Clinical Sciences Journal,* 9: 187-194.

Jacob, J.M., Olaleye, M.T. and Olugbuyiro, J.O. (2014). Hepatoprotective effect of *Alchornea cordifolia* leaf on liver damage in *albino* rats. *International Journal of Applied Science and Biotechnology,* 2(2): 217-221.

Jamuna, S., Subramanian, P. and Khrishnamoorthy, P. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herbs, *Hypochaeris radicata L.* for *in vitro* antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine,* 4(1): 359-367.

Jayathilakan, K., Sharma, G.K., Radhakrishna, K. and Bawa, A.S. (2007). Antioxidant potential of synthetic and natural antioxidants and its effect on warmed over-flavour in different species of meat. *Food Chemistry,* 105: 908–916.

Jendrassik, L. and Grof (1938). *In-vivo* determination of total and direct bilirubin in serum. *Journal of Biochemistry*, 299:81-88.

Jevas, C.O., Joseph, E.E., Obimba., K.C., Soniran, O. and Duru, M.J. (2014). Investigation of the antihepatoxic effects of *Allium sativium* extracts against acetaminophen intoxicated *rattus norvergicus.World Journal of Medical Sciences*, 11(3): 397-404.

Jude, C.I. (2012). An aqueous extract of the leaves of *Tridax procumbens* (Linn) (*Asteraceae)* protected against carbon tetrachloride-induced liver injury in wistar rats. *The Pacific Journal of Science and Technology,* 13(1): 519-527.

Jude, E.O., Michael, B.B. and Herbert, O.M. (2016). Hepatoprotective activity of *Mammea Africana* ethanol stem-bark extract. *Avicenna Journal of Phytomedicine*, 6(2): 248-259.

Kadiiska, M.B. and Gladen, B.C. (2005). Biomarker of oxidative stress study II: are oxidation products of lipids, proteins and DNA markers of CCl_4 poisoning?, *Free Radical Biology and Medicine,* 38:698-710.

Kala, C. P. (2007). Revitalizing Indian systems of herbal medicine by the natural medicinal plants board through institutional networking and capacity building. *Current Science,* 93(6): 797-806.

Karadag, A., Ozeelik, B. and Saner, S. (2009). Review of methods to determine antioxidant capacities. *Food Analytical Methods*, 2: 41-60.

Kaur, M. and Agarwal, R. (2007). Silymarin and epithelial cancer chemoprevention: how close we are to beside? *Toxicology and Applied Pharmacology*, 224: 350-359.

Khalid, G. A., Fathia, A. M. and Mosaad, A. A. (2013). The protective effects of whey protein and spirulina against CCl_4-induced erythrocyte damage in rats. *Journal of Applied Sciences Research,* 9(3): 2063-2071.

Khalid, R. (2007). Studies on free radicals, antioxidants and cofactors. *Clinical Intervention in Aging,* 2(2): 219-236.

Khan, M.R. and Siddique, F. (2012). Antioxidant effects of *Citharexylum spinosum* in CCl_4-induced nephrotoxicity in rat. *Experimental Toxicology and Pathology*, 64:349-355.

Khan, M.R., Marium, A., Shabbir, M., Saeed, N. and Bokhari, J. (2012). Antioxidant and hepatoprotective effects of *Oxalis corniculata* against carbon tetrachloride (CCl_4) induced injuries in rats. *African Journal of Pharmacy and Pharmacology,* 6(30): 2255-2267.

Kim, H.Y., Kim, J. K., Choi, J. H., Jung, J. Y., Oh, W. Y., Kim, D.C., Lee, H.S, Kim, Y.S., Kang, S.S., Lee, S.H., and Lee, S.M. (2010). Hepatoprotective effect of pinoresinol on carbon tetrachloride-induced hepatic damaged in mice. *Journal of Pharmacological science,* 112(1):105-112.

Kingsley, N.A., Moses, E.O. and Emmanuel, I.A. (2014). Phytochemical screening, hepatoprotective and antioxidant effects of leaf extracts of *Zapoteca portoricensis. Advances in Biological Chemistry,* 4: 35-39.

Kwazo, H.A., Faruq, U.Z., Dangoggo, S.M., Malami, B.S., and Moronkola, D.O. (2015). Antimicrobial activity and phytochemical screening of crude water extract of the stem-bark of *ficus glumosa*. *Scientific Research and Essays,* 10(5): 177-183.

Laetitia, K., Alan, B. and Catherine, R. (2012). Carbon tetrachloride mediated lipid peroxidation induces early mitochondrial alteration in mouse liver. *Laboratory Investigation*, 92: 396-410.

Lai, P.K. and Roy, J. (2004). Antimicrobial and chemoprotective properties of herbs and species. *Current Medical Journal,* 11(11): 1451-1560.

Le, T. (2013). First aid for the USMLE step 1, New York, MacGraw-Hill Medical 2013.

Levy, G.N. and Brabec, M.J. (1984). Binding of carbon tetrachloride metabolite to rat hepatic mitochondria DNA. *Toxicology Left,* 22: 229-234.

Li, W., Ming, Z., Yi-Nan, Z., Jing, L., Ying-Ping, W., Yun-Jing, W., Jian, G., Ying, J., Hui, W. and Li, C. (2011). Snailase Preparation of Ginsenoside M1 from Protopanaxadiol-Type Ginsenoside and Their Protective Effects against CCl_4-Induced Chronic Hepatotoxicity in Mice. *Molecules,*16: 10093-10103.

Lidia, R. and Wojciech, D. (2007). Application of silymarin in human and animal medicine. *Journal of Preclinical and Clinical Research,* 1(1): 2007

Lillie, R.D. (1965). Nuclei, nucleic acid, general oversight stains. *In*: Histopathology Technique and Practical Histochemistry, 3rd edition. Mc Graw Hill Book Company, Pp.142-179.

Lobo, V., Phatak, A. and Chandra., N. (2010). Free radical and functional foods: impact on human health. *Pharmacognosy Review,* 4: 118-126.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicol*ogy, Pp. 275-287.

Lu, J., Lin, P.H., Yao, Q., Chen, C. (2010). Chemical and molecular mechanisms of antioxidants: experimental approaches and model system. *Journal of Cellular and Molecular Medicine,* 14: 840-860.

Luminita, P., Ciprian, M., Hildegard, H., Cristina, P.,Violeta, T., Aurel, A., Ardelean, G., Teodora, M., Calin, P. and Anca, H. (2011). Pharmacology of *Silybum maramum* and its active constituents- therapeutic activity part-1. *Journal of Medical Aradean,* 4(2): 25-33.

Madubuike, K.G., Yusuf, N.O. and Ibekwe, A.M. (2015). Hepatoprotective activit of methanolic extract of *Jatropha tanjorensis,* in carbon tetrachloride induced hepatoxicity. *Archives of Applied Science Research,* 7(5): 45-48.

Maduka, H.C., Daja A., Okoye Gadaka G.A., Abubakar K.A. and Maduka A.A (2014). Protective role of moringa oleifera Lam aqueous extract on some excretory products and haematological parameters in acetamophen induced albino rats. *Journal of Nutrition and Health, 3(2): 27-31.*

Manibusa, M.K., Odin M. and Eastmond, D.A. (2007). Postulated carbon tetrachloride mode of action: a review. *Journal of Environmental Science Health Part C. Environmental carcinogen Ecotoxicity Review*, 5: 185-209.

Mariam, G. E., Hassenane, M.M., Ibrahim M. F., Nermeen, M. S. and Abodfetoh, M. A. (2015). Evaluation of protective and therapeutic role of moringa oleifera leaf extract on CCL4-induced genotoxicity, haematoxicity and hepatotoxicity in rats. *International Journal of PharmTech Research*, 7(2): 392-415.

Martin, J.P., Dailey, M. and Sugarman, E. (1987). Negative and Positive Assays of Superoxide Dismutase based on Heamatoxylin autoxidation. *Archives of Biochemistry and Biophysiology*, 255: 329-336.

Mates, D.M., Perez-Gomez, C. and DeCastro, I.N (1999). Antioxidant oxidant enzymes and human diseases. *Clinical Biochemistry,* 32: 595-603.

Maritim, A.C., Sanders, R.A. and Watkins III, J.B. (2003). Diabetes, oxidative stress and antioxidant. A review: *Journal of Biochemical and Molecular Toxicology,* 17(1): 2003.

Mecherey, A.C. and Dansette, P.M. (2008). Biotransformation leading to toxic metabolites: chemical aspect: In the practice of medical chemistry. Elsevier Publication, 2008, p682.

Mitchell, C., Robin, M.A. and Mayeuf, A. (2009). Protection against mitochondria dysfunction delays fibrosis progression in mice. *American Journal of Pathology,* 17: 1929-1937.

Mohajeri, D, Amouoghli, T. B., Doustar, Y. and Nazeri, M. (2011). Protective Effect of Turnip Root (*Brassica Rapa*. L) Ethanolic Extract on Early Hepatic Injury in Alloxanized Diabetic Rats. *Australian Journal of Basic and Applied Science*,5(7): 748-756.

Momoh, J., Longe, A.O., Damazio, O.A. and Eleyowo, O.O. (2015). Hepatoprotective effect of ethanolic leaf extract of *Vernonia amygdalina* and *Azadirachta indica* against acetaminophen-induced hepatotoxicity in sprague-dawley *albino* rats. *American Journal of Pharmacological Sciences,* 3(3):79-86.

Muhammad, A., Aisha, A., Azeem, M.A., Navia-ul-Zafar and Ahmad, S.I. (2013). Hepatoprotective effect of barrisal (herbal drug) on carbon tetrachloride induced hepatic damage in rats. *African Journal of Pharmacy and Pharmacology*, 7(15): 776-784.

Muriel, P. and Mourelle, M. (1990). The role of membrane composition in ATPases activities of cirrhotic rats. Effect of silymarin. *Journal of applied Toxicology*, 10: 281-284.

Muriel, P., Garciapina, T., Perez-Alvarez, V., (1992). Silymarin protects against paracetamol-induced lipid peroxidation and liver damaged. *Journal of Applied Toxicology*, 12 (6):439-442.

Nadro, M.S. and Onoagbe, I.O. (2014). Protective effects of aqueous and ethanolic extract of *Cassia italic* in CCl_4-induced liver damage in rats. *American Journal of Research Communication*, 2(6): 122-130.

Nitin, D., Sanjula, B., Kanchan, K., Ahmad, S. and Javed, A. (2007). Silymarin: a review of pharmacological aspects and bioavailability enhancement approaches. Indian Jouranl of Pharmacology, 39(4): 172-179.

Nwaoguikpe, R.N., Ujowundu, C.O., Emejulu, A.A., Iheme, C.I., Osam, M. and Oguoma, O.M. (2015). The ameliorative effect of aqueous leaves extract of *Acalypha wikessina* on carbon tetrachloride induced hepatotoxicity in wistar *albino* rats. *Journal of Current Research and Academic Review,* 3(9): 176-188.

Obioha, M.U., Ilodigwe, E.E., Ajaghaku, D.L. and Ume0koli, B.O. (2013). Hepatoprotective and antihepatotoxic activities of aqueous leaf extract of *Tacazzea barteri* against carbon tetrachloride-induced hepatotoxicity in *albino* rats. *International Research Journal of Pharmacy,* 4(9): 60-65.

Odutuga, A.A., Dairo, J.O.,Ukpanukpong, R.U. and Eze, F.N. (2014). Hepatoprotective activity of ethanol extracts of *Ficus exasperate* leaves on acetaminophen-induced hepatotoxic rats. *Merit Research Journal of Biochemistry and Bioinformatics,* 2(2): 28-33.

Ofeimun, J.O., Eze, G.I., Okirika, O.M. and Uanseoje, S.O. (2013). Evaluation of the hepatoprotective effect of methanol extract of the root of *Uvaria afzelli (Annonaceae).Journal of Applied Pharmaceutical Sciences,* 3(10): 125-129.

Olamide, E.A. and Mathew, O.A. (2013). Protective effect of *Enatia chlorantha* stem-bark extract on acetaminophen-induced liver damage in rats. *Jordan Journal of Biological Sciences,* 6(4): 284-290.

Olatosin, T.M., Akinduko, D.S. and Uche, C.Z. (2013). Evaluation of hepatoprotective efficacy of *Moringa oleifera* seed oil on CCl_4-induced liver damage in wistar *albino* rats. *International Journal of Engineering and Science,* 2(11): 13-18.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., and Anthony, A. (2009). Agroforestry databasea: A tree version 4.0, 2009, http//www.worldagroforestry.org.

Oyedepo, T.A. and Odoje, O.F. (2014). Hepatoprotective activities of tiger nut (*Cyperus esculentus*) against hepatotoxicity induced by carbon tetrachloride in rats. *Journal of Pharmacology and Toxicological Studies,* 2(4): 37-41.

Ozougwu, J.C and Eyo, J.E. (2014). Hepatoprotective effect of *Allium cepa* (onion) extracts against paracetamol-induced liver damage in rats. *African Journal of Biotechnology,* 13(26): 2679-2688.

Paglia, D.E. and Valentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidise. *Journal of Laboratory Clinical Medicine*, 70: 158-169.

Pallab, K., Tapan, B., Tapas, P. and Ramen, K. (2013). Estimation of total flavonoids content (TPC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. *Journal of Drug Delivery and Therapeutics,* 3(4): 33-37.

Patience, O.O., Festus, B.O., Philip, F.U., Nneka, R.N.,Ijeoma, E.A. and Nkemakonan, C.O. (2012). Phytochemical analysis, hepatoprotective and antioxidant activity of *Alchornea cordifolia* methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. *Asian Pacific Journal of Tropical Medicine,* 2012: 289-293.

Patil, S.B., Kodliwadmath, M.V., and Shela, M. K. (2007). Study of antioxidant stress and enzymatic oxidants inn normal pregnancy. *Iranian Journal of Clinical Biochemistry,* 22(1): 135-137.

Paul, O.A. (2013). Physicochemical properties and rheological behaviour of *ficus glumosa* gum in aqueous solution. *African Journal of Pure and Applied Chemistry,* 7(1): 35-43.

Pazos, M., Gallardo, J.M., Torres, J.L. and Medina, I. (2005). Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and fish muscle. *Food Chemistry*, 92: 547-557.

Polidori, M.C., Griffiths, H.R., Mariani E. and Mecocci P. (2007). Hallmarks of protein oxidative damage in neurodegenerative diseases: focus on Alzheimer's disease. *Amino Acids*, 32: 553-559.

Prochazkova, D., Bousova, I. and Wilhelmova, N. (2010). Antioxidant and proxidant properties of flavonoid. *Fitoterapia*, 82: 513-523.

Raheim, A.E., Donia, M., Gamal, A. S., Ahmad, M. Z., Saleh, I. A., Amani S. A., Asmaa M. R. and Omar, A. B. (2013). Chemical constituents and protective effect of *Ficus ingens* (Meq) on carbon tetrachloride-induced acute liver damage in male wistar albino rats. *Journal of Saudi Chemical Society*, 17: 125-133.

Rana, S.V., Sharma S., Prasad, K.K., Sinha, S.K., and Singh K. (2014). Role of oxidative stress and antioxidant difference in ulcerative colitis patients from north indian. *Indian Journal of Medicine Research*, 568-571.

Rasmasamy, G. and Agarwal, R. (2008). Multitargeted therapy of cancer by silymarin. *Cancer Letters,* 2008: 352-362.

Recknagel, R.O (1989). Mechanism of carbon tetrachloride toxicity. *Pharmacology and Therapeutics,* 43: 139-154

Reham, A., Mohamed, Reham, S., Ramadan and Lamiaa, A.A.(2009). Effect of Substituting Pumpkin Seed Protein Isolate for Caseinon Serum Liver Enzymes, Lipid Profile and Antioxidant Enzymes in CCl_4-intoxicated Rats. *Advances in Biological Research*, 3 (1-2): 09-15.

Saba, A.B., Oyagbemi A.A. and Azeez O.I. (2010). Amelioration of CCl_4-induced hepatotoxicity and haematotoxicity by aqueous leaf extract of *Cnidoscolus aconitifolius* in rats. *Nigerian Journal of Physiology Science,* 25: 139-147.

Salwa, H.N.A. and Abass K. A. (2011). And evaluation antioxidants and oxidative stress in Iraqi patients with thyroid gland dysfunction. *African Journal of Biochemistry Research*, 5(7): 188-196.

Samuel, O., Onoja, Y., Ndukaku, O., Maxwell, I. E., Emmanuel, C. O. and Destiny, S. E. (2014a). Subacute antidiabetic and in vivo antioxidant effects of methanolic extract of *Ficus glumosa* stem bark on alloxan-induced hyperglycaemic rats. *Comparative Clinical Pathology,* 23(6): 1689-1695.

Samuel, O.O., Yusuf, N.O, Maxwell, I.E. and Vincent, N.C. (2014b). hypolipidaemic and haematological evaluation of the hydromethanolic ectract of *Ficus glumosa* stem-bark in alloxan-inducced diabetic rats. *Journal of Complement Integration Medicine,* 11(4): 259-264.

Samuel, U., Innocent, N. and Obinna, O. (2011). Evaluation of hepatoprotective activity of aqueous leaf extract of *Swietenia mahogoni (maliaeceae)* in chronic alcohol-induced liver injury in rats. *Macedonian Journal of Medical Sciences,* 4(1): 31-36.

Sandra, L. S., Gemma, C., Kate, M. W. and Miguel, A. P. (2009). Chronic antioxidant therapy reduces oxidative stress in model of Alzheimer's disease. *Free Radical Research*, 43(2): 156-164.

Sanni, S., Thilza, I.B., Ahmad, M.T, Sanni, F.S., Muhammed, T. and Okwor, G.O. (2010). The effect of aqueous leaves extract of henna (*Lawsonia inermis)* in carbon tetrachloride induced hepatotoxicity in swiss *albino* rats. *Academia Arena,* 2(6): 87-89.

Sarah, O.N., Adeyinka, A.A. and Babatunji, E.O. (2012). Hepatoprotective effect of *Pipe guineense* againt ethanol induced toxicity im male rats. *Journal of Experimental and Integrative Medicine,* 2(1): 71-76.

Showkat, A. G., Ehtishamul, H., Abid, H., Yasrib, Q., Zahi M., Bilal, A. Z., Akbar, M. and Mahammad, A. Z. (2011). Carbon tetrachloride induced kidney and lung tissues damage and oxidative activities of aqueous rhizome extract of popophylum hexandrum. *BMC complementary and Alternative Medicine,* 11(17): 1472-6882.

Sreedevi, C.D., Latha, P.G., Aney, P., Suja, S.R., Shyamal, S., Shine, V.J., Sini, S., Anuja, G.I., and Rajasekharan, S. (2009). Hepatoprotective studies on *sida acuta* burm. F. *Journal of Ethnopharmacy*, 124: 171-175

Stein, E.A. (1987). Lipids, lipoproteins and apolipoproteins. In: Tietz N.W. (ed) Fundamentals of Clinical Chemistry. 3rd ed. W.B. Saunders, Philadelphia, PP. 470-479.

Subash, V. K., Saritha, G. and Md, F. (2010). Role of antioxidants and oxidative stress in cardiovascular diseases. *Scholar Research Library*, 1(3): 158-173.

Sylvester, C.O. (2015). Comparative analysis of hepatoprotective effect of ethanol extracts of *Gongronema latifolium* and *Alternanthera dentate* leaves in rats. *World Journal of Pharmacy and Pharmaceutical Sciences*, 5(1): 156-163.

Tanko, Y., Alladey, O., Ahmed, M.K., Mohammed, A. and Musa, K.Y. (2012). The effects of methanol leaves extract of ficus glumosa on gastrointestinal motility and on castor oil-induced diarrhoea in laboratory animals. *Jouranal of Nat. Prod Plant Resource.* 2: 360-367.

Tapsell, L.C., Hemphill, I. and Cobiac, L. (2006). Health benefits of herbs and species: the past, the present, the future. *Medical Journal,* 185(4): S4-S24.

Theophine, C. O., Peter, A.A., Adaobi, C.E., Maureen, O.O., Collins, A.O., Franklin, N., Ejike, O. and Lovelyn, I. (2012). Evaluation of acute and sub-acutetoxicity of *Annona senegalensis* root bark-extracts. *Asian Pacific of Tropical Medicine*, 2012: 277-282.

Thirupathi, G., Bhikku, A., Naguli, M., and Sandhya, G. R. (2011). Hepatoprotective effect of ageratum conyzoid against carbon tetrachloride intoxication in rats. *International Journal of Toxicology and Pharmacology,* 1(1): 21-26.

Trease, G.E. and Evans, W.C. (1983). Pharmacogonasy, 14th Edition, Brown Publication.

Ugwueze, M.E., Adonu, C.C. and Attama, A.A. (2013). Evaluation of the hepatoprotective activity of root extracts of *Millettia aboensis* on carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. *International Journal of Advanced Research,* 1(5): 65-70.

Ujah, O.F., Ipav, S.S., Anaebene, C.S. and Ujah, I.R. (2014). Phytochemical and hepatoprotective effect of ethanol leaf extract of *Corchorus olitorius* on carbon tetrachloride induced toxicity. *European Journal of Medicinal Plants,* 4(8): 882-892.

Umar, Z., Mohammed, A. and Tanko, Y. (2013). Effects of ethanol leaf extract of *Ficus glumosa* on fasting blood sugar and lipid profile in diabetic rats. *Niger Journal of Physiological Society,* 28: 99-104.

Umar, Z.U. and Mahaneem, M. (2015). The effect of ethanol extract of African *Ficus glumosa* in liver function in diabetic rats. *Journal of Molecular Pathophysiology,* 4(3): 103-107.

Usunobun, U. (2014). Anti-hepatotoxic efficacy of *Vernonia amygdalina* ethanolic leaf extract on dimethylnitrosamine (DMN)-induced liver damage in rats. *International Journal of Healthcare and Biomedical Research,* 3(1):89-98.

Uzzi, H.O. and Grillo, D.B. (2013). The hepatoprotective potentials of aqueous leaf extract of *Cassia occidentalis* against paracetamol-induced hepatotoxicity in adult wistar rats. *International Journal of Herbs and Pharmacological Research,* 2(2): 6-13.

Valenzuela, A., Aspillaga, M. and Vial, S. (1989). Selectivity of silymarin on the increase of the glutathione content in different tissues of the rat. *Planta Medica*, 55(5):420-422.

Vasudevan, D.M., Sreekumar, S.and Kanan, V. (2011). *Biochemistry for medical students*. Jaypee Brothers Medical Publishesr Limited. 6th edition. Pp147-159.

Venkat, V.L., Ranganathan, L. and Sindhi R., (2015). The challenges of the liver transplantation in children with sclerosing cholangitis. *Expert Review of Gastroenterology and Hepatology,* 9(3): 289-281.

Venkatanarayana, G., Sudhakara, P. and Sivajyothi, Pala Indira (2012).Protective Effects of Curcumin and Vitamin E on Carbon Tetrachloride-Induced Nephrotoxicity in Rats. *Experimental and clinical Journal,* 11: 641-650.

Ward, F.M., Daly, M.J., Rojer, W. and Clive, E. (2005). *Liver disease.* In clinical pharmacy and therapeutics. Churchill Livingstone, New York, p209.

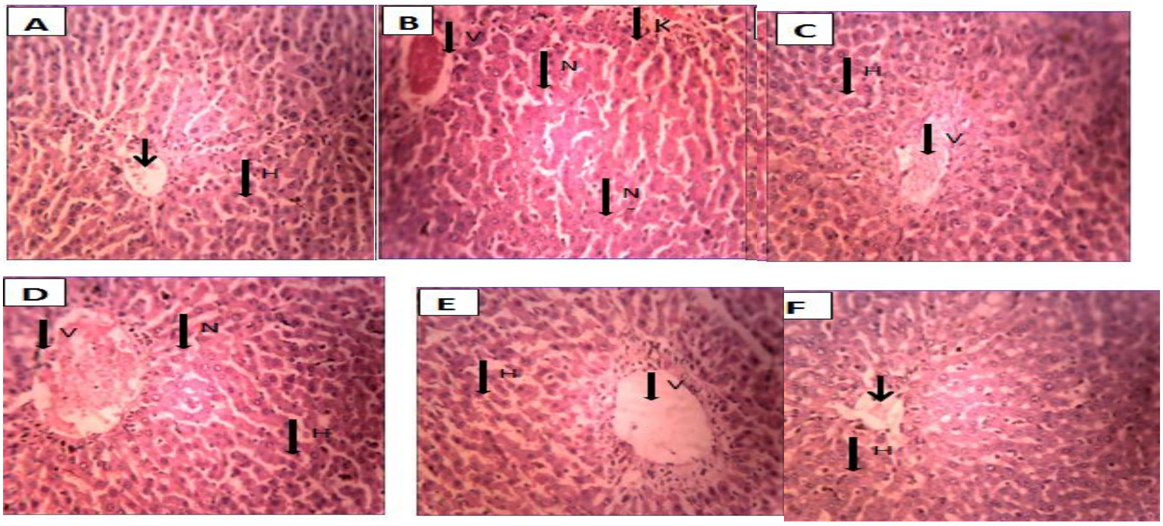
Weber, L.W., Boll, M. and Stampfl, A. (2003). Hepatoxicity and mechanism of action of haloalkanes: carbon tetrachloride as toxicological model. *Critical Reviews in Toxicology,* 3(3): 185-209.

Xia-wen, L., Rong, Z., Bo, L., Mei, Z., Quing-jian, S., Ye-peng, Y., Nan-yan, H., and Zai-quan, L. (2010). Mechanism underlying carbon tetrachloride-inhibited protein synthesis in the liver. *World Journal of Gastroenterology,* 16(31): 3950-3956.

Yacout, G. A., Elguindy, N.M. and El-Azab, E. F. (2012).Hepatoprotective Effect of Basil (*Ocimum Basilicum* L.) On CCl_4-Induced Liver Fibrosis in Rats. *African Journal of Biotechnology,* 11 (90): 15702-15711.

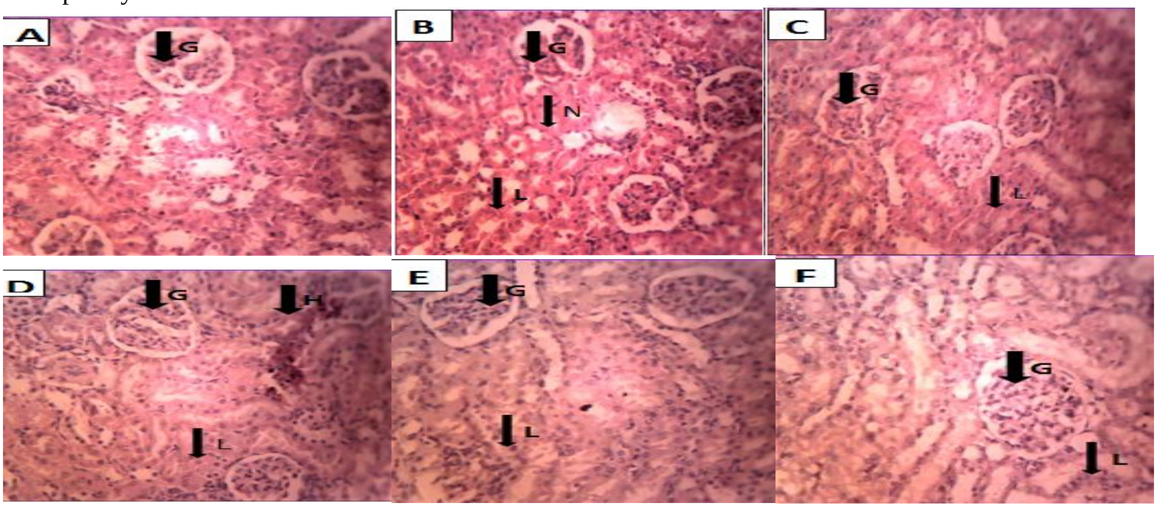
APPENDIX 1.0

Representaive Photomicrograph of Liver and Kidney of CCl_4-induced Liver Damaged *Albino* Rats



(H&E STAIN X250)

Plate I: Representaive photomicrograph of liver of CCl_4-induced liver damaged *Albino* Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves (A) Normal control group (B) 1ml/kg CCl_4-induced but not treated (C) 100 mg/kg of Sylimarin group (D). 100 mg/kg of *F. glumosa* extract (E) 300 mg/kg of *F. glumosa* extract (F) 500 mg/kg of *F. glumosa*. V=vascular congestion, N=necrosis, K=kuffer cells, H=hepatocytes



(H&E STAIN X250)

Plate II: Representaive photomicrograph of kidney of CCl_4-induced liver damaged *Albino* Rats Treated with *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves (A) Normal control group (B) CCl_4-induced (1ml/kg) (C) 100 mg/kg of Sylimarin group (D). 100mg/kg of *F. glumosa* extract. (E). 300 mg/kg of *F. glumosa* extract (F). 500 mg/kg of *F. glumosa* extract. G=glomerulus, N=Necrosis, L=lymphocyte

APPENDIX 2.0

Samples and Reagents Preparations

Preparation of 20% Na_2CO_3

20g of Na_2CO_3 --------------- 100ml of H_2O

Xg of Na_2CO_3 ---------------- 50ml of H_2O

Xg = 20g X 50ml/100ml = 10g

Accurately 10g of Na_2CO_3 was dissolved in 50ml of distilled H_2O to give 20% Na_2CO_3

Preparation of 5% Na_2NO_3

Exactly 5g of Na_2CO_3 was dissolved in 100ml of distilled H_2O

Preparation of 10% $ALCL_3$

Accurately 10g of $AlCl_3$ was dissolved in 100ml of distilled H_2O

Preparation of 1M NaOH

Mass = molarity X molar mass

Molar mass of NaOH = 40

Mass = 1 X 40 = 40g

40g --------------------- 1000ml of distilled H_2O

Xg --------------------- 100ml of distilled H_2O

Xg = 40 X 100/1000 = 4g

NaOH (4g) was dissolved in 100ml of distilled H_2O to give 1M of NaOH

Preparation of Folin-Ciocalteu's reagent

Exactly 10g of sodium tungstate and 2.5g of sodium molybdite were dissolved in 70ml of distilled H_2O. Exactly (5ml) of 85% of phosphoric acid and 10ml of concentrated HCL acid were added. The mixture was reflux for 10 hours. Then, 15g of lithium sulphate, 5ml of distilled H_2O and 1 drop of bromine were added. The mixture was further reflux for 15 minutes and cool to room temperature and the volume was them made up to 100ml with distilled H_2O.

Preparation of Varying Concentrations of Gallic Acid Standard Solution

Gallic acid solution stock (20µg/ml): Exactly 1mg was dissolved in 50ml of distilled H_2O. Serial dilution was made to obtain different concentrations of the stock solution (0.625, 1.25, 2.5. 5, 10, 20 µg/ml).

Preparation of Varying Concentrations of Quercetin Standard Solution

Quercetin solution stock (60µg/ml): Exactly 3mg was dissolved in 50ml of ethanol. Dilutions were made to obtain (10, 20, 30, 40, 50, 60 µg/ml).

Preparation of Varying Concentrations of L-Ascorbic Acid Standard Solution

L-Ascorbic acid solution (10mg/ml): Exactly 100mg was dissolved in 10ml of distilled H_2O. Serial dilution was made to obtain different concentrations of the stock solution (0.625, 1.25, 2.5. 5, 10, 20 mg/ml).

Preparation of Extract Fractions

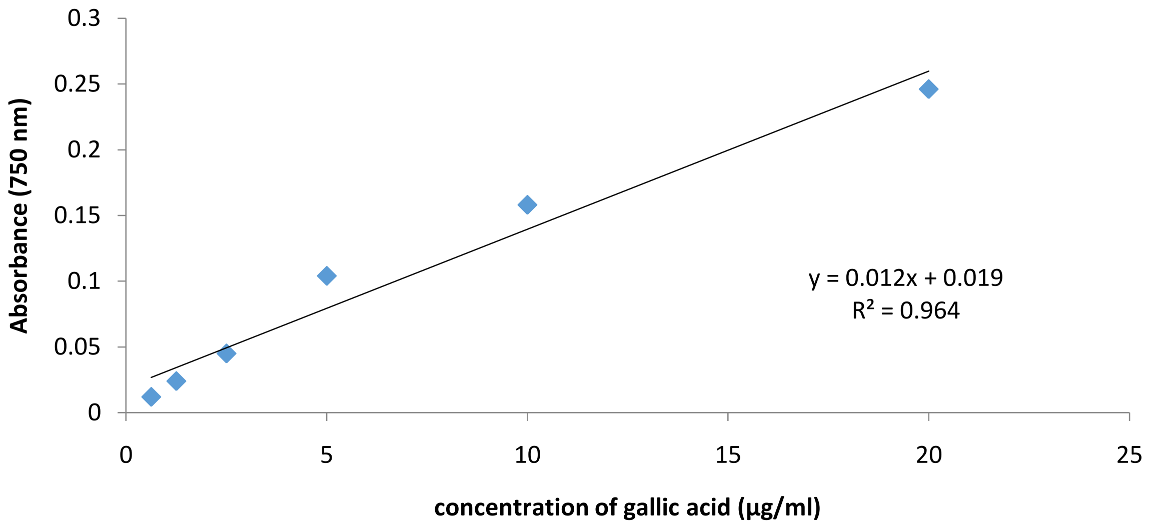
1g of extract fractions were dissolved in 5ml of distilled H_2O.

APPENDIX 3.0
Estimation of Total Phenols, Flavonoids and Ascorbic Acid

Construction of Calibration Curve using Gallic Acid to Determine Ascorbic acid in the Extracts

Concentration (µg/ml)	Mean
0.625	0.012
1.250	0.024
2.500	0.045
5.000	0.104
10.00	0.158
20.00	0.246

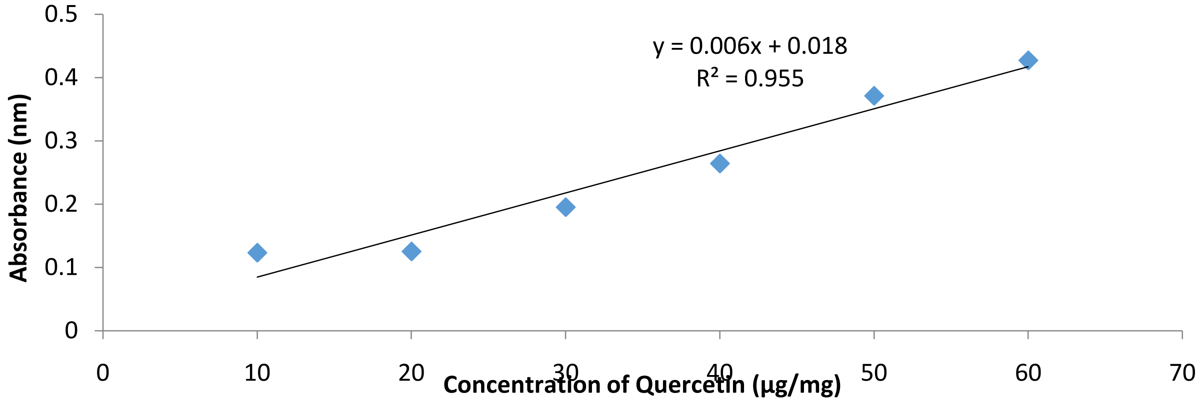
Values are in mean of triplicate observation of absorbance



Construction of Calibration Curve using Quercetin to Determine Flavonoids in the Extracts

Concentration (µg/ml)	Mean Absorbance (nm)
10	0.123
20	0.125
30	0.195
40	0.264
50	0.371
60	0.427

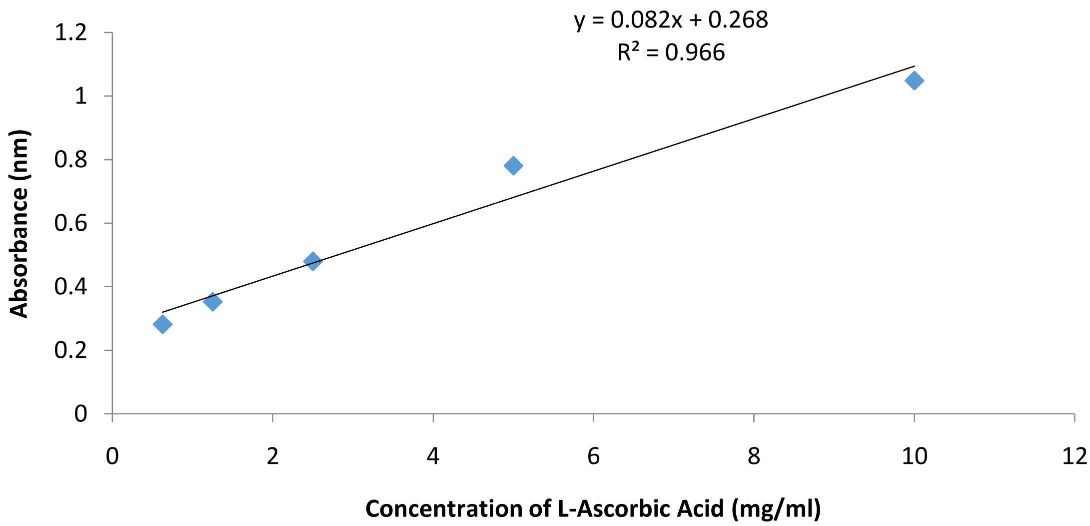
Values are in mean of triplicate observation of absorbance



Construction of Calibration Curve using L-Ascorbic to Determine Ascorbic acid in the Extracts

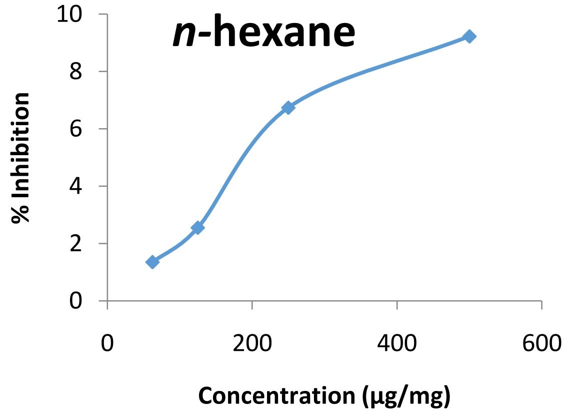
Concentration (mg/ml)	Mean Absorbance (nm)
0.625	0.281
1.25	0.352
2.5	0.479
5	0.78
10	1.048

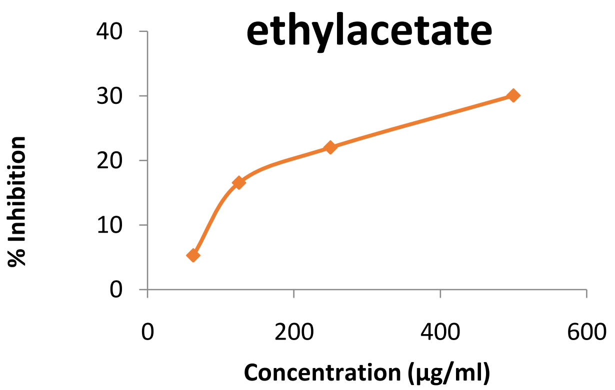
Values are in mean of triplicate observation of absorbance

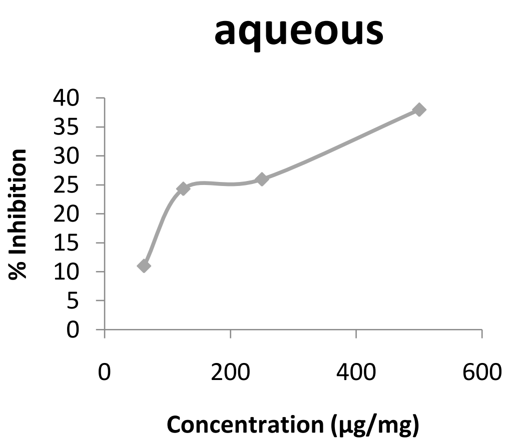


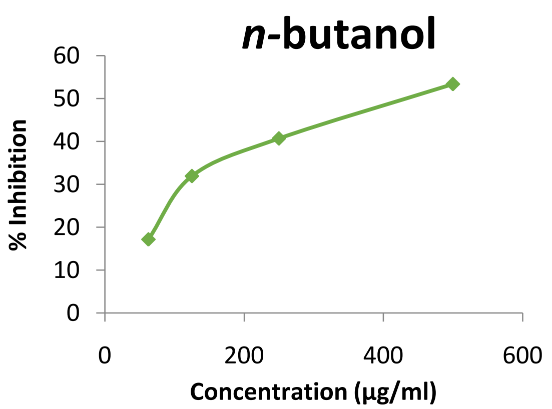
Expressing of sample concentration in terms of Gallic acid and quercetin equivalent
GAE or QE (mg/g) = C X V/M; Where C = concentration from standard curve V = Volume of the sample used M = Mass of the sample

APPENDIX 4.0
Deduction of IC_{50} by DPPH Scavenging Activity









Plot of % inhibition against concentration to deduce IC_{50}; Values plotted are mean of triplicate observations