ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Hepatoprotective activity of aqueous and ethanol extract of *Lippia adoensis* leaf against carbon tetrachloride-induced hepatotoxicity in mice

Yared Wasihun (B. Pharm)

A Thesis Submitted to the School of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology in the Department of Pharmacology, School of medicine.

Addis Ababa, Ethiopia

March 2015

Hepatoprotective activity of aqueous and ethanol extract of *Lippia adoensis* leaf against carbon tetrachloride-induced hepatotoxicity in mice.

Advisor: Professor Eyasu Makonnen

Department of Pharmacology, School of Medicine, College of Health Science, Addis Ababa University

Co-advisors: Dr.Mekbeb Afewerk

Department of Anatomy, School of Medicine, College of Health Science, Addis Ababa University

Dr.Wondwosen Ergete

Department of Pathology, School of Medicine, College of Health Science, Addis Ababa University.

A Thesis Submitted to the School of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology in the Department of Pharmacology, School of medicine.

Investigator

Yared Wasihun (B. Pharm)

March 2015

Acknowledgments

I wish to express my profound gratitude to my advisor; Professor Eyasu Makonnen for his unreserved guidance, constructive suggestions and comments from the very conception, development and finalization of this thesis. My sincere gratitude also goes to Co-advisors Dr.Wondwosen Ergete and Dr.Mekbeb Afewerk for their valuable advice and follows up throughout the course of my work.

I would like to thank the Department of Medical Pharmacology, AAU graduate school for sponsoring to pursue my study.

I would take this opportunity to extend my thanks to all Department of Pharmacology, school of medicine, laboratory staffs for their help and encouragement through the research process.

Thanks to almighty God, the giver of knowledge, and my ever present help in time of need for making this possible.

Table of Content Pages

Acknowledgments i

Table of Content iv

List of Table vii

List of Figure viii

Abbreviation ix

Acronyms viii

Abstract ix

1. Introduction 1

1.1 Drug and toxin induced hepatic injury 2

1.2 Acute liver injury 4

1.3 Pathphysiological conditions related to liver diseases 5

1.3.1 Oxidative stress 6

1.3.2 Effect of oxidative stress 6

1.3.3 Oxidative protein damage 7

1.3.4 Antioxidant defence system 7

1.4 Pro-oxidant activity of flavonoids 9

1.5 Hepatoprotective effects 10

1.6 Role of herbal medicines against liver disease 10

1.7 Lippia adoensis 11

2. Objective of study 13

General objective 13

Specific objectives 13

3. **Materials and method** 14

3.1 Chemicals and Reagents 14

3.2 Collection of plant materials 14

3.3 Preparation of plant extracts 14

3.4 Experimental Animals 15

3.5 Experimental design 15

3.5.1 Limit dose test 15

3.5.2 Hepatoprotective activity 16

3.5.3 Blood Collection 16

3.5.4 Serum Biochemical Analysis 16

3.5.5 Histopathological examination 17

3.5.6 Ethical considerations 17

3.5.7. Statistical analysis 17

4. **Results** 18

4.1 Yields of the aqueous and 80% ethanol Extracts 18

4.2 Effect of the leaves extracts of Lippia adoensis on weights of the body and liver of mice 18

4.2.1 Effects of *Lippia adoensis* extract on body weight change, Liver weight and relative liver weights ratio in CCl_4 intoxicated mice. 18

4.3 Serum Biochemical Analysis 20

4.3.1 Effects of *Lippia adoensis* extract on the serum biochemical parameters 20

4.3.1.1 Effects of *Lippia adoensis* extract on AST, ALT, and ALP Levels. 21

4.3.1.2 Effects of *Lippia adoensis* extract on total bilirubin, albumin and total Protein levels. 22

4.4 Effect of *Lippia adoensis* extracts on Histopathology 25

5. **Discussion** 28

6. **Conclusion** 33

7. **Recommendation** 34

8. **References** 35

List of Table Pages

Table 1: Percentage yields of the aqueous and 80% ethanol extracts of the dried and powdered leaves of *Lippia adoensis*. 18

Table 2: Effects of *Lippia adoensis* leaves extracts on body weight change, and absolute and relative liver weights 19

Table 3: Effects of extracts of *Lippia adoensis* on CCl_4 induced liver damage in mice 24

1.

List of Figure Pages

Figure 1: Section of liver from the control group showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100). 26

Figure 2: Liver section from mice treated with CCl_4 at a dose of 2 mg/kg;bw showinga focal mononuclear infiltration (arrow),H&E staining (x100). 26

Figure 3: Liver section from mice treated with silymarin and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100). 26

Figure 4: Liver section from mice treated with 200 mg/kg *Lippia adoensis* aqueous extractandCCl_4 showing normal architecture of the hepatic lobule with minimal focus of mononuclear infiltration at the periphery (arrow) H&E staining (x100). 26

Figure 5: Liver section from mice treated with 400 mg/kg *Lippia adoensis* aqueous extract and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100). 27

Figure 6: Liver section from mice treated with 200 mg/kg *Lippia adoensis* ethanolic extract and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100). 27

Figure 7: Liver section from mice treated with 400 mg/kg *Lippia adoensis* ethanolic extract and CCl_4showingnormal architecture of the hepatic lobule with no histopathological change H&E staining(x100). 27

Abbreviation

ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CCl_4	Carbontetrachloride
dl	Decilitter
g	Gram
GPx	Glutathione peroxidase
Kg	Kilogram
L	Liter
Mg	Milligram
Mol.wt	Molecular weight
P.o	Per os
Trx	Thioredoxin
TrxR	Thioredoxin reductase

Acronyms

AAU	Addis Ababa university
bw	Body weight
EPHI	Ethiopian Public Health Institute
H&E	Haematoxylin and Eosin
IU	International unit
OECD	Organization of Economic Co-Operation Development
PUFA	Polyunsaturated fatty acid
RNS	Reactive nitrogen species
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
RPM	Round per minute
SEM	Standard errors of mean
SOD	Superoxide dismutase

Abstract

Medicinal plants may be the best source of remedies for the treatment of liver disease. Thus identification of a potential therapeutic agent for protection of liver from hepatotoxins provides a useful way for the prevention of liver related illnesses. The aim of the present study was to investigate the hepatoprotective activity of 80% ethanolic and aqueous extract of leaves of *Lippia adoensis* against carbontetrachloride in mice.

Mice were pretreated with extract of *Lippia adoensis* (200 & 400 mg/kg bw.po) for 7 days and then challenged with CCl_4 (2ml/kg bw po.) on the 7^{th} day. Serum biomarkers (AST, ALT, ALP and total bilirubin, total protein and albumin) were estimated in all the study groups. Alteration in the levels of biochemical parameters of hepatic damage like AST, ALT, ALP and total bilirubin, total protein and albumin were tested in both CCl_4 and *Lippia adoensis* extract treated groups. CCl_4 significantly ($P<0.001$) increased the AST, ALT, ALP and total bilirubin levels and decrease the level of total protein and albumin. Pretreatment with the extract of *Lippia adoensis* (200 & 400 mg/kg) produced significant ($P<0.001$) hepatoprotective effects as evidenced by decreased serum enzymes (AST, ALT, ALP) and serum bilirubin and increase the level of total protein and albumin as well as by histopathological findings of the liver. Histopathological examination of the liver tissues of CCl_4 group represented the presence of marked foci of mononuclear infiltration in the hepatic parenchyma tissue, sinusoid and around central vein, as well as disorganization of hepatic plates, while the pretreatment with extract of *Lippia adoensis* overcome most of these changes to normal histological architecture of the liver in treated groups, compared to the controls. Change in the body weight of *Lippia adoensis* extract treated groups increased significantly ($P < 0.001$) as compared to carbontetrachloride treated group. The absolute and relative liver weights of extracts treated mice decreased significantly ($P < 0.001$) as compared to those treated with CCl_4. Both ethanolic and aqueous extract of *Lippia adoensis* were comparable with the standard drug, Silymarin. The experimental results indicate that, ethanolic and aqueous extract of *Lippia adoensis* has potential hepatoprotective effect.

Key words: *Lippia adoensis*, Hepatoprotective, CCl_4, Silymarin, AST, ALT, ALP, Bilirubin, Protein and Albumin, Ethanol extracts, Aqueous extract, Hepatotoxicity, mice.

1. Introduction

Liver is the largest organ in the body and plays a significant role in protecting various biological function and help in detoxification and excretion of various endogenous and exogenous compounds (Mohamed *et al*., 2010).

The basic structure of the liver consists of rows of hepatic cells perforated by specialized blood capillaries called sinusoids. The sinusoid walls contain phagocytic cells called Kupffer cells whose role is to engulf and destroy materials such as solid particles, bacteria, dead blood cells, etc. The main blood supply comes to the liver from the intestinal vasculature. These vessels merge with each other to form the portal vein. On entering the liver, the portal vein subdivides and drains into the sinusoids. The blood then perfuses the liver and exits by the hepatic veins, which merge into the inferior vena cava and return blood to the heart **(**Rajeev *et al*., 2012).

The liver is often the target organ for chemically induced injuries. Many oxidative reactions produce reactive metabolites that can induce lesions within the liver. The types of injury to the liver depend on the type of toxic agent, the severity of intoxication, and the type of exposure, whether acute or chronic (Comfort *et al*., 2013; Singh *et al*., 2011).

Liver is a pivotal inflammatory organ that, involved in metabolism, storage, and excretion of metabolites (Guan *et al*., 2012). Inflammation is a complex, vascular and cellular reaction by the immune system to either external or internal injurious agents such as pathogens, irritants or other noxious stimuli characterized by pain, redness, swelling and sometimes loss of function (Shanmugam *et al*., 2012).

Acute inflammatory response is characterized by vasodilation, leakage of the vasculature and infiltration of leukocytes into the site of infection to destroy invading pathogens and is followed by a rapid resolution phase and repair of the damaged tissue (Kundu and Surh, 2012).

1.1 Drug and toxin induced hepatic injury

There are considerable numbers of hepatotoxins that have been reported to cause liver damage, such as tetracycline, salicylates, yellow phosphorus and ethanol induced microvasicular fattychange, ethanol, methotrexate and amiodarone induced macrovasicular fatty change, bromobenzene, CCl_4, acetaminophen, halothane and rifampin induced centriolobular necrosis (Sanjay *et al*., 2014).

Diffuse or massive necrosis are induced by halothane, isoniazid, acetaminophen, methyldopa,trinitrotoluene and amanitaphallodies (mushroom) toxin, hepatitis, acute and chronic are induced by methyldopa, isoniazid, nitrofurantoin, phenytoin,oxyphenisatin. Fibrosis-cirrhosis is induced by ethanol, methotrexate and amiodarone; most drugs that cause chronic hepatitis.cholestasis (with or without hepatocellular injury) are induced by chlorpromazine, anabolic steroids, erythromycin, oral contraceptives and organic arsenicals (Mary *et al*., 2010).

Upon stimulation from various hepatotoxins, kupffer cells which release proinflammatory mediators such as NO and Interferon-gamma (IFN-γ) will eventually result in accumulation of reactive oxygen species. Reactive oxygen species have been shown to cause lipid peroxidation and membrane degradation which will generate liver damage and inflammation **(**Mohamed *et al*., 2010).

Hepatic injury is associated with distortion of various metabolic functions. It is well known that reactive oxygen and nitrogen species play a crucial role in initiation and progression of liver associated diseases such as alcoholic and viral hepatitis, non- alcoholic steatosis, cholestasis and hepatocellular carcinoma **(**Halina and Agata , 2014).

The progression of liver fibrosis may develop into cirrhosis and is associated with liver cancer. Nearly 10–20% of patients progress to cirrhosis which further leads to increasing the risk of hepatocellular carcinoma **(**Michael, 2014).

Steroids, vaccines, and antiviral drugs have been employed for treatment of liver diseases which have adverse effects if administrated for long term. Extensive studies reported that natural

products with antioxidant activity are effective to prevent the oxidative stress related liver pathologies due to particular interactions and synergisms (Chao *et al*., 2013; Eman *et al*., 2014).

Hepatic fibrosis occurs during most chronic liver diseases and is driven by inflammatory responses to the injured tissue **(**Magda *et al*., 2011). Human and animal studies suggest that hepatic immunity is altered in fibrosis and that liver inflammation is the hallmark of early stage liver fibrosis (Georg *et al*., 2013).Various immunoregulatory cytokines such as tumor necrosis factor-α (TNF-α) is a critical mediator in fibrosis **(**Jayashree *et al*., 2012).Considering the hazards of treatment failure, drug resistance and heavy costs associated with current hepatic therapy, there is strong interest in the study of natural compounds with protecting and free radicals scavenging capacity (Azza *et al*., 2013). Medicinal plants, especially those with traditional use have always been considered as a rich source of antioxidants **(**Street *et al*., 2013).

Drug-induced liver disease occurs in several different clinical presentations; idiosyncratic reactions, allergic hepatitis, toxic hepatitis, chronic active toxic hepatitis, toxic cirrhosis, and liver vascular disorders **(**Robert *et al*., 2014).

The mechanisms of drug-induced liver disease are diverse, representing many phases of biotransformation, and are susceptible to genetic polymorphism (Michael and Cynthia, 2006). Liver enzyme assays can help determine if a particular type of liver damage is present. monitoring for drug induced liver disease must be tailored to the drug and the patient's potential risk factors. Several synthetic and natural plants have been proved effective in hepatic tissue protection due to their antioxidant property **(**Areefa *et al*., 2012).

Chemical toxicity comprises an important source of reactive oxygen species (ROS), which may occur through processes, such as inhibition of mitochondrial electron transport chain and subsequent accumulation of intermediates inactivation of antioxidant enzymes and deletion of radical scavengers **(**Singh *et al*., 2011**).**

Carbontetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease. Carbontetrachloride is commonly used as a model to evaluate hepatotoxicity. Carbontetrachloride metabolism begins with the formation of the trichloromethyl free radical, $CCl_3^{\cdot}$through the action of the mixed function cytochrome P450 oxygenase system of the

endoplasmic reticulum (Akram *et al*., 2012). The CCl_3 radical reacts with various biologically important substances such as amino acids, nucleotides and fatty acids, as well as proteins, nucleic acids and lipids. In the presence of oxygen, the CCl_3 radical is converted to the trichloromethyl peroxy radical ($CCl_3OO^{\cdot}$). This radical is more reactive and is capable of abstracting hydrogen from polyunsaturated fatty acids (PUFA) to initiate the process of lipid peroxidation. Modulation of cellular thiols has been used to protect the hepatocytes against attack by reactive oxygen intermediates and is currently being investigated as a novel therapeutic strategy in different liver pathologies (Russel *et al*., 2014).

1.2 Acute liver injury

Acute liver injury is the most common form of liver injury caused by drugs, accounting for approximately 10 percent of all cases of acute hepatitis (Michael *et al.*, 2014). The patterns of acute injury may include cholestasis, hepatocellular damage, a mixed pattern of cytotoxic and Cholestatic injury or less commonly, steatosis (Raúl *et al*., 2007). Discontinuation of the offending agent usually results in complete recovery, although the prognosis is generally worse in patients with hepatocellular injury presenting with jaundice (Abhinav *et al*., 2014). In the setting of cholestatic injury, jaundice can take months to resolve. Acute liver injury is probably the most common cause of cholestatic hepatitis (Michael *et al*., 2014).

Drug-induced acute hepatocellular injury is similar to that seen in viral hepatitis. Laboratory testing characteristically shows an elevation in serum aminotransferases. Histologically, portal and parenchymal hepatocellular injury leads to acute hepatocellular necrosis or apoptosis, steatosis, and/or cellular degeneration (David *et al*., 2014). A hepatocyte that has become sensitized to the immune system dies by apoptosis triggered via death receptors at the cell surface. Moderate degrees of oxidative stress also result in apoptosis at the intracellular level, while severe oxidative stress leads to necrosis (Richard and Andreas, 2008).

Hepatocellular injury can be affecting single hepatocytes, or groups of hepatocytes. Groups of hepatocytes necrosis can be zonal or nonzonal, depending upon the offending agent. If extensive enough, groups of hepatocytes necrosis results in acute liver failure (David *et al*., 2014).

Cytotoxic hepatocellular injury is associated with a mortality rate of up to 10 percent overall and up to 80 percent or higher if acute liver failure develops (Ali Canbay and Guido, 2014). The best predictor of mortality in the setting of acute hepatocellular injury is a serum bilirubin level greater than three times the upper limit of normal (Abhinav *et al*., 2014). Acute cholestatic injury often resembles extrahepatic obstructive jaundice. Cholestatic injury is typically recognized by predominant elevations in alkaline phosphatase, γ-glutamyl transferase, and bilirubin (Michael *et al*., 2014).

1.3 Pathphysiological conditions related to liver diseases

The pathophysiology of liver disease has been linked to different factors, such as oxidative stress, inflammation and apoptosis. Oxidative stress has been recognized to be an important feature of many acute and chronic liver diseases and even the normal aging process (Montuschi *et al*., *2004).*

Oxidative stress is a consequence of an imbalance between free radical generation and antioxidant capacity of the cell and increasing evidence has emerged pointing to a causal link between oxidative stress and liver disease states. Several reports have shown that oxidative stress is closely linked with cardiovascular diseases and coronary disease risk factors such as endothelial dysfunction, myocardial ischaemic injury, atherosclerosis, hypertension and cancers (Sekar *et al*., 2013; Ambar *et al*., 2011) .

Oxidative stress causes a complex dysregulation of cell metabolism and cell-cell homeostasis and the emerging concept based on the study of several diseases, i.e., it is the final converging pathway through which risk factors of several diseases exert their deleterious effects (Pitocco *et al*., 2010).

Scientific evidence has indicated that inflammation and oxidative stress are inseparably interconnected as inflammatory processes induce oxidative stress and deplete cellular antioxidant capacity (Khansari *et al*., 2009).

Several studies have confirmed the strong relationship between inflammation and acute liver disease (Luiz-Rodriguez *et al*., 2012). Nuclear Factor-Kappa B (NF-κB) has been implicated as a

major mediator of inflammation in most chronic disease conditions and it has been established that its inhibition can prevent and delay the onset of chronic diseases (Aggarwal *et al*., 2012).

1.3.1 Oxidative stress

Oxidative stress has been defined as an imbalance between the pro-oxidant and antioxidant steady state in the cell, with the excess of pro-oxidants being available to interact with cellular macromolecules to cause damage to the cell, often resulting in cell death (Small *et al*., 2012).

Free radicals target macromolecules in their proximity for their electrons, thereby oxidizing them and generating other free radicals. If the macromolecules targeted are important parts of the cellular structure, such as nucleic acid, proteins and lipids, considerable oxidative injury can occur (Halliwell and Gutteridge, 2007).

Free radicals are classified into two groups; reactive oxygen species and reactive nitrogen species and both often act together to create a state of oxidative stress either as a result of depletion of cellular antioxidant defense molecules or over-production of the reactive species (Small *et al*., 2012).

1.3.2 Effect of oxidative stress

Oxidative attack by reactive oxygen species and reactive nitrogen species is manifested as damage to nucleic acid bases, lipids, and proteins, which can severely compromise cell function and viability or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis (Sarah *et al.*, 2012).

Macromolecular damage via oxidative stress, if unchecked, can theoretically contribute to disease development and an increasing amount of evidence suggests that oxidative stress is linked to the pathophysiologic mechanisms of a myriad of human diseases (Klaunig *et al*., 2010; Small *et al*., 2012).

1.3.3 Oxidative protein damage

Proteins are primarily responsible for most functional processes within cells and are thus highly abundant in biological systems, making them important targets of reactive oxygen species and reactive nitrogen species attack. The attack of reactive oxygen species and reactive nitrogen species on the polypeptide backbone is initiated by an •OH-dependent abstraction of the α-hydrogen atom from an amino acid residue to form a carbon-centered radical, which under aerobic conditions readily reacts with molecular oxygen to form peroxyl radicals, which reacted with the protonated form of superoxide (HO2•) and are converted to the alkyl peroxides (Valko *et al.,* 2007).

Attack by reactive oxygen species and reactive nitrogen species on proteins may lead to the oxidation of amino acid residue side chains, as well as oxidation of the protein backbone and formation of protein-protein cross-linkages. Consequently, protein fragmentation and generation of many protein oxidation products which can cause damage to other biomolecules may occur (Pandey and Rizvi, 2010).

Oxidized proteins are usually degraded by proteosomal and lysosomal pathways, and since degradation of damaged proteins are not completely efficient, functionally inactive proteins that are poorly degraded may form high molecular mass aggregates which accumulate with age in separate compartments within cells or in the extracellular environment (Seifert *et al*., 2010; Avery, 2011).

1.3.4 Antioxidant defence system

Under normal circumstances, eukaryotic cells have evolved a defence mechanism to limit free radicals and the damage caused by them. These include systems based on the presence of antioxidant molecules, repair of injured molecules and removal of damaged molecules. The antioxidant defence system can be endogenous and/or exogenous (Punitha and Rajasekaran, 2011).

The endogenous system is made up of a network of antioxidant enzymes including superoxide dismutase(SOD),catalase (CAT), glutathione peroxidase(GPx) and the glutaredoxin and peroxiredoxin system as well as low molecular weight antioxidant molecules such as vitamin E (major membrane bound antioxidant),vitamin C (ascorbic acid,) (major aqueous phase antioxidant), uric acid, glutathione and ceruloplasmin (Kang and Koppula , 2014).

Superoxide dismutase catalyses (SOD) the dismutation of superoxide anion, converting it to molecular oxygen and H_2O_2.There are three isoforms of the SOD family and they all utilize a transition metal at their active site. There is a CuZn SOD form in the cytosol and the intermembrane mitochondrial compartment, a Mn SOD in the mitochondrial matrix and another form in the extracellular compartment (e.g. blood) (Muhammad khan *et al.,* 2014).

Though, less reactive than the O_2•−anion, the H_2O_2 must still be rapidly removed and this can be accomplished by the enzymes catalase and peroxidase working coordinately. Catalase remove H_2O_2 at a high rate but show low affinity for the peroxide, thus it should be most useful during the peak of H_2O_2 production or accumulation (Pamplona and Costantini, 2011).

Glutathione peroxidases utilize the reducing power of GSH (and other thiols, such as thioredoxin) to decompose H_2O_2 and it is the sulfhydryl moiety of the cysteine residue that supplies the reducing equivalent for peroxidase activity. Two molecules of GSH are oxidized for every one molecule of H_2O_2 decomposed, resulting in the formation of GSSG which can be re-reduced back to two molecules of GSH by glutathione reductase (Al Meheithif *et al*., 2014).

Various low molecular weight endogenous non-enzymatic antioxidants are found in animal and human tissues. These are usually depleted when they react with reactive oxygen species and reactive nitrogen species, but are actually recycled back to the antioxidant form due to reduction by other molecules. Because of their low molecular weight, they are able to eliminate reactive oxygen species at sites that much larger enzymes cannot access (Mehwish and Syeda, 2014).

Reduced glutathione (GSH), thioredoxin and ascorbate are the main low molecular weight hydrophilic non-enzymatic antioxidant molecules in the cell (Al Meheithif *et al.,* 2014). Glutathione (L-γ-glutamyl-L-cysteinylglycine) is the predominant intracellular non-protein thiol in eukaryotic cells. It possesses strong antioxidative properties and consequently plays a crucial

role in intracellular protection against compounds such as reactive oxygen species and reactive nitrogen species and other free radicals (Imran *et al*., 2014).The thioredoxin system is another major intracellular antioxidant system and comprise of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH. It is an oxidoreductase with a redox-active disulfide/dithiol at the active site (Hashemy, 2011).

The other most abundant reduced non-enzymatic antioxidant in cells is ascorbate. It is endogenously synthesized and maintained at high levels in tissues, but in primates (including human), guinea pigs, fruit-eating bats and many bird species, it is obtained through diet. Ascorbate can scavenge RONS and other free radicals by readily donating an electron to potentially damaging radicals, such as hydroxyl (OH•), alkoxyl (RO•), peroxyl (LOO•), thiol radical (GS•) and tocopheroxyl radicals (TO•) becoming to the ascorbate radical ($Asc^{\bullet -}$) during the process (Du *et al*., 2012).

1.4 Pro-oxidant activity of flavonoids

Pro-oxidant activities of dietary antioxidants, such as carotenoids or polyphenols have also been reported. It has been suggested that the antioxidant and pro-oxidant properties of flavonoids could be important in determining the fate of a cell, with the biological response, beneficial or deleterious, depending on the prevailing oxidative status in the cell (Joubert *et al*., 2005).

Evidence has shown that the antioxidant and pro-oxidant activity of flavonoids depends on certain factors, including metal-reducing potential, chelating ability, pH and solubility characteristics (Sakihama *et al*., 2002). Polyphenols are oxidized to produce superoxide anion radicals, hydrogen peroxide and a complex mixture of semiquinones and quinones, which are all potentially cytotoxic (Lambert *et al.*, 2007; Halliwell, 2007).

Some reports have suggested that a pro-oxidant effect of flavonoids can be beneficial, because imposing a mild oxidative stress, might induce an up regulation of antioxidant defenses and xenobiotic metabolising enzymes, resulting in overall cytoprotection (Tang and Halliwell, 2010). Also the pro-oxidant effect of flavonoids has been proposed as one of the mechanisms for the anti-cancer function of most flavonoids (Zhang *et al*., 2012).

The reactive oxygen species oxidative stress generated by these flavonoids have been reported to play important role in inhibition of tumor cell growth and induction of apoptosis, which eliminates cancer cells (Lambert and Elias, 2010).

1.5 Hepatoprotective effects

Chronic hepatic disease represents one of the foremost health problems worldwide, with liver cirrhosis and drug-induced liver injury accounting for the ninth leading cause of death in the western and developing countries (Saleem *et al*., 2010). It is a well known fact that the available synthetic drugs to treat liver disorders might also cause further damage to the liver (Chen *et al*., 2011).

Hence herbal medicines have become increasingly popular and their use is widespread. Hepatoprotective effects of medicinal plants have been reported in a number of studies. Consumption of medicinal plants protected against liver damage by suppressing the observed increase in plasma activities of AST,ALT, alkaline phosphatase, bilirubin, and increasing protein and albumin, resulted in a histological regression of cholestasis, steatosis and cirrhosis in the liver of mice challenged with carbon tetrachloride (2 mL/kg, p.o) (Sharma , 2010).

1.6 Role of herbal medicines against liver disease

Medicinal plants contain antioxidants that might serve as leads for the development of novel drugs. The use of natural remedies for the treatment of liver diseases have a long history, starting with the ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or anti-radical scavenging mechanism as part of their activity (Lambert *et al.,* 2010; Kumari ., 2014).

In search of natural antioxidants and compounds with radical scavenging activity during recent years, some have been found, such as *curcumin and ginger* (Magda et al., 2011), *Hibiscus* species (Mohamed *et al*., 2014), *polyalthia longifolia* (Balamuruganvelu *et al*., 2014) and *Cassia italica* (Nadro *et al*., 2014). Antifibrotic effect of *Monochoria.vaginalis* in hepatic disorders (Bhaskara and Mukalel, 2014).

One of the most extensively studied agents is Silybum marianum, commonly known as ‘milkthistle’Family:Asteraceae/Compositae) is one of the oldest and thoroughly researched plants in the treatment of liver diseases. The active constituents of the plant are obtained from the dried seeds and consist of four flavonolignans which are collectively known as silymarin. Silymarin, a single herbal drug formulation which is mostly used in liver diseases (Ramadan *et al.*, 2011).Amongst the flavonoids, which have proven antioxidative,antiviral or anticarcinogenic properties like glycyrrhizin, phyllanthin, silybin, picroside and baicalein, can serve as primary compounds for further development as hepatoprotective drugs (Nancy Vargas *et al*., 2014).

1.7 *Lippia adoensis*

Lippia adoensis is included under Verbenaceae that is a large family with about 70 to 80 genera and over 3,000 species; distributed throughout the world mainly in the tropics and temperate regions. *Lippia* is a genus with 200 species in tropical Africa and America (Toshihiro *et al*., 1992).

In Ethiopia, the family is represented by 9 genera and 30 species. *Lippia adoensis* (locally known as “Kesse”) is a shrub having a height of 1 to 3 meters. Two varieties of *Lippia adoensis* are recognized in Ethiopia, the wild variety (var.*adoensis*) and the cultivated variety (var.*koseret*). The fragrant leaves are used by the Gurage and Oromo tribes as one of the condiments in the preparation of spiced butter. The special taste and flavor of the “Gurage Kitfo” is attributed to the oils imparted by the leaves **(**Toshihiro *et al*., 1992**).** In Ethiopia, the wild variety (var. *adoensis*) is used for washing kitchen utensils to impart fresh and spicy fragrance (Abegaz *et al*., 1993).

Scientific studies dealing with the pharmacological activities of this particular species is nonexistent except one study that investigated the antioxidant activity of the volatile oil and the major terpenoids therein. The result indicated that the essential oil possess a significant radicals scavenging property when assessed in the DPPH (diphenylpicrylhydrazyl) assay. As free radical oxidative stress is implicated in the pathogenesis of a variety of human diseases including inflammation, the traditional uses of the plant for the treatment of various kinds of inflammatory skin diseases is partly justified **(**Abegaz *et al*., 1993).

The chemical compositions of *Lippia adoensis*, investigated so far are essential oils. The oils were predominantly monoterpenoids with minor sesquiterpenoid fraction. In one study, out of more than 10 compounds isolated, linalool comprised 81.30% and 94.56% of the leaf and flower oils, respectively (Asres and Bucar, 2002).

The leaves of *L. adoensis* are used in Ethiopian traditional medicine for the treatment of various skin diseases including eczema and superficial fungal infections (Asres *et al*., 1986). There are some data about the hepatoprotective activity of *Lippia nodiflora* (Sudha *et al*., 2013). Review of literature revealed that this rare medicinal plant remained unexplored for many of its claimed pharmacological activities. The present study was conducted to establish the use of *Lippia adoensis* as hepatoprotective against CCl_4 induced hepatotoxicity in mice.

2. Objective of study

General objective

To investigate the hepatoprotective activity of aqueous and ethanol extract of *Lippia adoensis* leaf in carbon tetrachloride induced hepatotoxicity in mice.

Specific objectives

- To investigate the effect of the extracts on body weight change and liver weight in mice.
- To evaluate the effect of the extracts on histopathologic changes in liver of mice.
- To investigate the effects of the extracts on the following serum biochemical parameters in mice:
    - Alanine aminotransferase (ALT)
    - Aspartate aminotransferase (AST)
    - Alkaline phosphate (ALP)
    - Total bilirubin
    - Total protein
    - Albumin

3. Materials and method

3.1 Chemicals and Reagents

Silymarin (Silybon-140) was purchased from MICRO LAB LIMITED, INDIA (ML13F-0140) and used as standard drug. CCl_4, Ethanol, 10% formalin, ether, normal saline, paraffin wax. Hematoxylin, eosin, xylol**,** DPX and All chemicals were obtained from local sources and were of analytical grade.

3.2 Collection of plant materials

Fresh leaves of *L.adoensis* were collected in Addis Ababa, Ethiopia, on January 18, 2014. Plant identification and authentication was done with the help of local floras. The specimen has been preserved in AAU herbarium with the voucher number of 084908.

The fresh leaves of *L.adoensis* was dried under shed, and powdered with a mechanical grinder. The powdered plant material was then sieved with sieve No 40, and stored in an air tight container for future use.

3.3 Preparation of plant extracts

One kilogram of dried powder plant material was macerated in ethanol (80%) in a ratio of 1:1.The ethanolic extract was filtered with filter paper (Whatman No 3, Whatman Ltd., England). The filtered extract was then concentrated by evaporation using Rota vapor at temperature not exceeding 40 ^{o}C. Then the aqueous residue was placed in an oven for about 48 hours to remove the water under reduced pressure at a temperature of 40 ^{0}C. The resulting powder was stored in to amber colored glass bottle in desiccators over silica gel for use in subsequent experiments.

One kilogram of dried powder plant material was macerated in water in a ratio of 1**:**1.The mixture was allowed to stand and infuse with continuous shaking. The mixture was then filtered using filter paper (Whatman No 3, Whatman Ltd., England). The filtrate was lyophilized for 24 h to obtain freeze-dried powder. The resulting dried masses was converted to powder then, packed into amber colored glass bottle and stored in desiccators over silica gel until use.

3.4 Experimental Animals

A total of 42 healthy male mice weighing 25-35 gram were obtained from animal house of department of pharmacology, School of medicine, AAU and *Ethiopian Public Health Institute (EPHI)* were used throughout the study. They were kept under standard environmental conditions at 22±3°C with 12**:**12 hour light–dark cycle in ventilated plastic cages. The animals were housed in steel mesh cages, (6 per cage) and had a free access to feed with a standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experimentation.

3.5 Experimental design

3.5.1 Limit dose test

Limit test was performed following OECD (Organization of Economic Co-Operation Development) guidelines – 423. The purpose of this study was to allow selection of the appropriate starting dose for the main study.

Limit test of *L.adoensis* was performed in female mice. The mice were kept fasting for overnight prior to experimentation, and body weight of the mice was noted. Mice weighting 25-35 gram were used for limit dose test. The dose was given to every mouse orally according to body weight. Limit dose test was performed at 2000 mg/kg, and 3000mg/kg oral dose of Ethanolic and aqueous leaves extract of *L.adoensis*. After administration of *L.adoensis* extract, food was withheld for further 2 hour.

During the first four hour after administration of *L.adoensis* extract, mice were continuously observed for gross behavioral changes & then observation is continued for 24 hr & 72 hr in regular intervals for 14 days. Hyperactivity, convulsions, sedation, hypothermia, and change in fur colour, mortality, moribund stage or death were observed. The animals did not show any signs of toxicity and behavioral changes after 24 hrs and 72 hrs. The oral LD_{50} was estimated to be above 3000mg/kg.

3.5.2 Hepatoprotective activity

The body weights of all mice were recorded at the beginning of the experiment. Experimental mice were divided in seven groups (n-7) of six mice/ group. The animals were fasted overnight before the initiation of the study. Group I (Control) served as normal control which received vehicle (5% gum acacia; 1 ml/kg; p.o) orally daily for seven days. Group II (CCl_4) served as toxicant control and received CCl_4 (0.2 ml/100 gm p.o) on seventh day. Group III served as standard and received silymarin (100 mg/kg p.o) daily for seven days and thirty minutes post dose of silymarin mice received CCl_4 (0.2 ml/100 gm p.o.) on the seventh day. Group IV and Group V were treated with the aqueous extract of *L.adoensis* at the doses of 200 mg/kg and 400 mg/kg, respectively in 5% aqueous gum acacia orally daily for seven days. Group VI and Group VII were treated with the ethanolic extract of *L.adoensis* at the doses of 200 mg/kg and 400 mg/kg, respectively in 5% aqueous gum acacia orally daily for seven days. Thirty minutes post dose of extract administration, animals received CCl_4 at the dose of 2 ml/kg orally (Sunita *et al.*, 2014; Sabreena *et al*., 2014**;** Showket *et al*., 2014).

3.5.3 Blood Collection

Twenty four hour after administration of acute dose of CCl_4, The animals were weighed and sacrificed under light ether anesthesia. The blood sample from each mouse was collected separately in sterilized dry centrifuge tubes by cardiac puncture and allowed to coagulate for 10 minutes at 37 ^{0}C.The clear serum was separated at 2500 rpm for 10 minutes and subjected to biochemical estimations like aspartate aminotransferase(AST),alanine aminotransferase (ALT), alkaline phosphate (ALP) ,albumin, total bilirubin and total protein using diagnostic kits (Maliha and AL-Marzooq, 2014; Iyanda and Adeniyi, 2014).

3.5.4 Serum Biochemical Analysis

Liver biochemical tests (AST, ALT, and ALP) were performed on a clinical chemistry automatic analyzer ADVIA 2400 (Bayer Diagnostics). AST, ALT, and ALP were measured according to the method of Reitman and Frankel (Reitman and Frankel, 1957; Young *et al*., 1975) using commercial assay kit (Bayer Diagnostics). The serum total concentrations of total protein,

albumin, and Bilirubin were measured according to the methods of (Gornall *et al.*, 1949 and Doumas *et al.,* 1971) using commercial kits on the Express Plus biochemical analyzer (Ciba-Corning Diagnostics).

3.5.5 Histopathological examination

After collection of blood sample, the mice were scarified and then the liver was excised from the animals and washed with normal saline. The tissues of liver were fixed in 10% formalin. Formalin fixed liver tissues were washed in tap water, dehydrated in serial ethanol**,** cleared in xylene and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome from the paraffin embedded block and stained with haematoxylin and eosin for histopathological observations. The microscopic slides were examined under a microscope by a pathologist who was blind to the protocol of the study (Alaezi *et al*., 2014; Bhaskara and Mukalel, 2014).

3.5.6 Ethical considerations

The experiment was carried out in accordance with ethical principles for laboratory use and care in animal research (Bernard, 2004).

3.5.7. Statistical analysis

The results were expressed as means ± standard errors of mean (SEM).The data of hepatoprotective activity were analyzed by one way analysis of variance (ANOVA). P<O.05 was considered statistically significant. The statistical analysis was carried out using the SPSS program (version 21.0).

4. Results

4.1 Yields of the aqueous and 80% ethanol Extracts

Leaves of *Lippia adoensis* were extracted with aqueous and 80% ethanol using maceration technique and these extracts were used for investigation of hepatoprotective activity. The percentage yields of these extracts are given in Table (1). The yields of aqueous and ethanol extracts were found to be 16.3% and 12.7%, respectively. The yields obtained from the leaves of *Lippia adoensis* were quite adequate to carry out the study.

Table 1: Percentage yields of the aqueous and 80% ethanol extracts of the dried and powdered leaves of *Lippia adoensis*.

Plant species	Extracts	Parts used	Percentage yield
Lippia adoensis	Aqueous Extracts	Leaves	16.3
Lippia adoensis	80% Ethanol Extracts	Leaves	12.7

4.2 Effect *of* the leaves extracts of *Lippia adoensis* on weights of the body and liver of mice

Table (2) shows effect of the leaves extracts of *Lippia adoensis* on the body weight, liver weight and relative liver weight (%) of mice.

4.2.1 Effects of *Lippia adoensis* extract on body weight change, Liver weight and relative liver weights ratio in CCl_4 intoxicated mice.

In the present study, body weight change of the experimental groups treated with *Lippia adoensis* extract increased significantly ($P < 0.001$) as compared to carbontetrachloride treated group. Both the absolute and relative weights of the liver of extracts treated mice decreased ($P < 0.001$) as compared to those treated with CCl_4.

The body weight change of mice treated with hepatotoxins (CCl_4) significantly ($P < 0.001$) decreased, as compared to the control mice. The absolute and relative liver weights of mice treated with CCl_4 significantly increased ($P < 0.001$) as compared to those of the control mice, increased ($P<0.001$) as compared to silymarin treated mice.

Table 2: Effects of *Lippia adoensis* leaves extracts on body weight change, and absolute and relative liver weights.

Groups Treatment	**Body weight (gm)**		**Body weight Change**	**Liver weight (gm)**	**Relative liver Weight (%)**
	Initial	**Final**			
Control	**29.00 ± 0.93**	**32.33 ± 0.88**	**2.83 ± 0.60**	**1.47±0.05**	**4.53±0.084**
CCl_4 2ml/kg	**32.17±0.74***	**28.33 ± 0.76****	**-3.50 ±0.61#**	**2.32±0.06#**	**7.97±0.43#**
Sylimarin100mg/kg	**29.83 ±0.48*****	**32.50±0.43****	**2.67±0.33****	**1.50±0.03****	**4.52±=0.06****
Aqueous extract200mg/kg	**28.83 ± 0.79***	**30.83±0.65*****	**1.83±0.30****	**1.76±0.06****	**5.53±0.23****
Aqueous extract400mg/kg	**28.33 ± 0.67***	**30.67±0.56*****	**2.33± 0.49****	**1.69±0.07****	**5.50±0.28****
Ethanolic extract200mg/kg	**27.83 ± 0.83****	**30.67±0.88*****	**2.83±0.40****	**1.71±0.10****	**5.62±0.42****
Ethanolic extract400mg/kg	**29.17±0.7031***	**31.83±0.83***	**2.83±0.48****	**1.65±0.16****	**5.20±0.57****

Values are expressed as means ± standard error of means (SEM) of six mice treated for **7** days.

* P <0.01, ** P < 0.001, # P < 0.001, *** P <0.05 were statistically significant.

Symbols represent statistical significance; **#** significantly different from control

****** Significantly different from CCl_4 treated group**.**

4.3 Serum Biochemical Analysis

4.3.1 Effects of *Lippia adoensis* extract on the serum biochemical parameters

The effects of leaves extract of *Lippia adoensis* on the serum transaminases, alkaline phosphatase, bilirubin, total protein and albumin levels in carbon tetrachloride induced liver damage in mice are summarized in Table(3). Carbon tetrachloride (2 mg/kg; bw) produced significant ($P < 0.001$**)** elevation of serum biochemical markers ALT, AST, ALP, bilirubin and significant ($P < 0.001$**)** reductions in serum total protein and albumin concentrations.

Administration of 200 mg/kg and 400 mg/kg of aqueous extract of *Lippia adoensis* reduced the mean ALT values to 143.67±2.44 IU/L ($p<0.001$) and 138.33±3.29 IU/L ($p<0.001$), respectively which were comparable to the standard silymarin 129.83±2.02 IU/L ($p<0.001$). Administration of 200 mg/kg and 400 mg/kg of ethanolic extract of *Lippia adoensis*, reduced the mean ALT values to 141.83±2.76 IU/L ($p<0.001$) and 140.50±2.01 IU/L ($p<0.001$), respectively which were comparable to the standard silymarin 129.83±2.02 IU/L ($p<0.001$).

The normal mean value of AST 104.17± 4.41 IU/L was elevated to 251.83±3.46 IU/L with carbontetrachloride (2 mg/kg; bw) pretreatment which was reduced to 135.50±4.27 IU/L ($p<0.001$) and 127.0±1.79 IU/L ($p<0.001$) with doses of 200 mg/kg and 400 mg/kg of aqueous extract, respectively.AST reduction was also comparable to that of silymarin control 112.50±3.24 IU/L ($p<0.001$). Administration of 200 mg/kg and 400 mg/kg of ethanolic extract of *Lippia adoensis* reduced the mean AST values to 128.83±2.89 IU/L ($p<0.001$) and 126.17±4.38 IU/L ($p<0.001$), respectively which were comparable to the standard silymarin 112.50±3.24 IU/L ($p<0.001$).

The normal mean value of ALP (201.17±4.52 IU/L) increased after administration of carbon tetrachloride (2 mg/kg; bw) to 446.50±3.67 IU/L. Administration of 200 mg/kg and 400 mg/kg aqueous extract brought the enzyme value of ALP down to 257.83±2.75 IU/L ($p<0.001$) and 225.5±2.44 IU/L ($p<0.001$), respectively; while 200 mg/kg and 400 mg/kg of the ethanol extract reduced it to 233.67±3.45 IU/L ($p<0.001$) and 227.67±3.05 IU/L ($p<0.001$,) respectively which were comparable to the standard silymarin 220.67±2.95 IU/L ($p<0.001$).

The mean total bilirubin value in mice was raised from 1.15±0.13 to 4.48 ±0.12 mg/dl after carbon tetrachloride (2 mg/kg; bw) administration. When these animals were treated with 200 mg/kg and 400 mg/kg of the aqueous extract the total bilirubin reduced to 2.05±0.14 mg/dl ($p<0.001$) and 1.98 ±0.25 mg/dl ($p<0.001$), respectively. Administration of 200 mg/kg and 400 mg/kg of ethanolic extract of *Lippia adoensis* reduced the total bilirubin to 2.22±0.11mg/dl ($p<0.001$) and 2.10 ±0.13 mg/dl ($p<0.001$), respectively which were comparable to that of the standard silymarin 1.17±0.07 mg/dl ($p<0.001$).

Of the total mean protein value in mice was reduced from 6.75± 0.21 to 4.63±0.15 g/dl after carbon tetrachloride (2 mg/kg; bw) administration. When these animals were treated with 200 mg/kg and 400 mg/kg of aqueous extract, the total protein raised to 6.17±0.11 g/dl ($p<0.001$) and 6.23±0.09 g/dl ($p<0.001$), respectively. Administration of 200 mg/kg and 400 mg/kg of ethanolic extract of *Lippia adoensis* raised the total protein to 6.15±0.11 g/dl ($p<0.001$) and 6.22±0.08 g/dl ($p<0.001$), respectively which were comparable to that of standard silymarin 6.67±0.12 g/dl ($p<0.001$).

The mean value of albumin in mice was reduced from 2.18 ±0.15 to 1.67±0.20 g/dl after CCl_4 administration. When these animals were treated with 200 mg/kg and 400 mg/kg of aqueous extract, the albumin raised to 2.62±0.09 mg/dl ($p<0.001$) and 2.63±0.07 mg/dl ($p<0.001$), respectively. Administration of 200 mg/kg and 400 mg/kg of ethanolic extract of *Lippia adoensis* raised the albumin to 2.65±0.085 mg/dl ($p<0.001$) and 2.67±0.11 mg/dl ($p<0.001$,) respectively which were comparable to that of the standard silymarin 2.47±0.17 mg/dl ($p<0.001$).

4.3.1.1 Effects of *Lippia adoensis* extract on AST, ALT, and ALP Levels.

The levels of AST, ALT, and ALP in serum were significantly ($P< 0.001$) increased in carbon tetrachloride treated mice (251.83±3.46, 286.83±2.52 and 446.50±3.67IU/L), respectively, as compared to the AST, ALT, and ALP of control mice (104.17± 4.41, 126.67±1.61and 201.17±4.52 IU/L), respectively.

After administration of carbon tetrachloride to the *Lippia adoensis* ethanolic extract (200mg/kg) treated mice, the levels of AST, ALT, and ALP were significantly (P<0.001) reduced (128.83±2.89, 141.83±2.76 and 233.67±3.45 IU/L), respectively. In ethanolic extract (400mg/kg) treated mice, the levels of AST, ALT, and ALP were significantly (P<0.001) reduced to 126.17±4.38, 140.50±2.01 and 227.67±3.05 IU/L, respectively. After administration of carbon tetrachloride to the *Lippia adoensis* aqueous extract (200mg/kg) treated mice, the levels of AST, ALT, and ALP were significantly (P<0.001) reduced to 135.50±4.27, 143.67±2.44 and 257.83±2.75 IU/L, respectively.

In *Lippia adoensis* aqueous extract (400mg/kg) treated mice, the levels of AST, ALT, and ALP were significantly (P<0.001) reduced to 127.0±1.79, 138.33±3.29 and 225.5±2.44IU/L, respectively. Administration of the standard drug, silymarin also significantly (P < 0.001) reduced these levels (112.5±3.46, 129.83±2.02, 220.67±2.95 IU/L,) respectively. Thus the results showed that *Lippia adoensis* extract at the doses of 200 and 400mg/kg bw reduced the levels of AST, ALT, and ALP levels comparable to the standard drug, Silymarin.

4.3.1.2 Effects of *Lippia adoensis* extract on total bilirubin, albumin and total Protein levels.

The level of total bilirubin in serum was significantly (P<0.001) increased in carbon tetrachloride treated control group (4.48 ±0.12 mg/dl) compared to the total bilirubin of control mice (1.15±0.13 mg/dl). *Lippia adoensis* ethanolic extract (200mg/kg) reduced the levels of total bilirubin significantly (P < 0.001) to 2.22±0.11 mg/dl. In *Lippia adoensis* ethanolic extract (400mg/kg) treated mice, the levels of total bilirubin were significantly (P<0.001) reduced to 2.10 ±0.13 mg/dl. *Lippia adoensis* aqueous extract (200mg/kg) reduced the levels of total bilirubin significantly (P<0.001) to reduced 2.05±0.14 mg/dl. In *Lippia adoensis* aqueous extract (400mg/kg) treated mice, the levels of total bilirubin were significantly (P<0.001) reduced to 1.98 ±0.25mg/dl. Administration of standard drug, silymarin also significantly (P<0.001) reduced the total bilirubin level to 1.17±0.07mg/dl. The level of total protein was significantly (P<0.001) reduced in carbon tetrachloride treated mice to 4.63±0.15 gm/dl. Administration of 200mg/kg and 400mg/kg of *Lippia adoensis* ethanolic extract; significantly (P<0.001) increased the total protein level to 6.15±0.11 and 6.22±0.08 gm/dl, respectively.

Aqueous extracts (200mg/kg and 400mg/kg) significantly (P<0.001) increased the total protein level to 6.17±0.11and 6.23±0.09 gm/dl, respectively. Whereas standard drug, silymarin also increased the level to 6.67±0.12 gm/dl. Thus the results showed that 200 and 400mg/kg; bw of *Lippia adoensis* extract reduced the bilirubin and total protein levels comparable to the standard drug silymarin.

The level of albumin was significantly (P<0.001) reduced in carbon tetrachloride treated mice to 1.67±0.20 mg/dl when compare to control mice (2.18 ±0.15 mg/dl).After administration of 200mg/kg and 400mg/kg;bw of *Lippia adoensis* ethanolic extracts, the level of albumin increased; significantly (P<0.001) to 2.65±0.085 and 2.67±0.11 mg/dl, respectively, when compared to the carbon tetrachloride treated animals (no treatment) (1.67±0.20 gm/dl).

Aqueous extracts (200mg/kg and 400mg/kg) significantly (P <0.001) increased albumin level to 2.62±0.09 and 2.63±0.07 mg/dl, respectively. Standard drug, Silymarin significantly (P < 0.001) increased the level to (2.47±0.17 mg/dl).Hence the results showed that the *Lippia adoensis* extract at the doses of 200 and 400mg/kg bw increase albumin levels comparable to the standard drug, Silymarin**.**

Table 3: Effects of extracts of *Lippia adoensis* on CCl_4 induced liver damage in mice

Sr. no	Group	Dose (po)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Protein (gm/dl)	Total Bilirubin mg/dl)	Albumin mg/dl
1	Control	Vehicle(5% Acacia)	104.17± 4.41	126.67±1.61	201.17±4.52	6.75± 0.21	1.15±0.13	2.18 ±0.15
2	CCl_4 control	2ml/kg	251.83±3.46*	286.83±2.52*	446.50±3.67*	4.63±0.15*	4.48 ±0.12*	1.67±0.20*
3	Silymarin + CCl_4	100mg/kg	112.50±3.24**	129.83±2.02**	220.67±2.95**	6.67±0.12**	1.17±0.07**	2.47±0.17**
4	Aqueous extract + CCl_4	200mg/kg	135.50±4.27**	143.67±2.44**	257.83±2.75**	6.17±0.11**	2.05±0.14**	2.62±0.09**
5	Aqueous extract + CCl_4	400mg/kg	127.0±1.79**	138.33±3.29**	225.5±2.44**	6.23±0.09**	1.983±0.25**	2.63±0.07**
6	Ethanolic extract + CCl_4	200mg/kg	128.83±2.89**	141.83±2.76**	233.67±3.45**	6.15±0.11**	2.22±0.11**	2.65±0.085**
7	Ethanolic extract + CCl_4	400mg/kg	126.17±4.38**	140.50±2.01**	227.67±3.05**	6.22±0.08**	2.10 ±0.13**	2.67±0.11**

Values are mean ± S.E.M, (n=6); $P < 0.001$

Symbols represent statistical significance * considered significantly different at $P < 0.001$ when compared with normal control group; ** considered significantly different at $P < 0.001$ when compared with CCl_4 alone control group.

4.4 Effect of *Lippia adoensis* extracts on Histopathology.

Histopathological analysis revealed that normal control group showed normal histology of the liver. The liver sections of the control group exhibited, normal hepatic cells, visible central veins and thin sinusoids (Figure 1). In contrast carbon tetrachloride (2 ml/kg**;** bw) intoxication caused marked foci of mononuclear infiltration in the hepatic parenchyma tissue, sinusoid and around central vein, as well as disorganization of hepatic plates. There was massive necrosis throughout the liver, in some areas whole lobules being degenerated, in others the more central hepatic cells showing the greater disintegration (Figure 2). Pretreatments with *Lippia adoensis* extracts at different concentrations (200 and 400mg/kg bw) resulted in normal hepatic cells, central vein and sinusoids with no necrosis and foci of mononuclear infiltration. The treatment with standard drugs silymarin also showed normal hepatic architecture (Figure 3).

In the liver section from 200 mg/kg *Lippia adoensis* aqueous extract and CCl_4 treated mice hepatic cells, central vein and sinusoids were normal, except the presence of a minimal focus of mononuclear infiltration at the periphery (Figure 4). Furthermore, in the liver section from 400 mg/kg *Lippia adoensis* aqueous extract and CCl_4 treated mice there was a marked protection with normal hepatic cells, central vein and sinusoids (Figure 5). Similarly, in the liver section from 200 mg/kg *Lippia adoensis* ethanolic extract and CCl_4 treated mice there was a marked protection with normal hepatic cells, central vein and sinusoids (Figure 6)**.**

There was also no histopathological change in the sections of the liver from 400 mg/kg *Lippia adoensis* ethanolic extract and CCl_4 treated mice with normal hepatic cells, central vein and sinusoids (Figure 7).The histological appearance of the *Lippia adoensis* extract pretreated groups was quite similar to that of the control group, and tissue damage and necrosis were of less extent in *Lippia adoensis* extract treated group than the CCl_4 group.

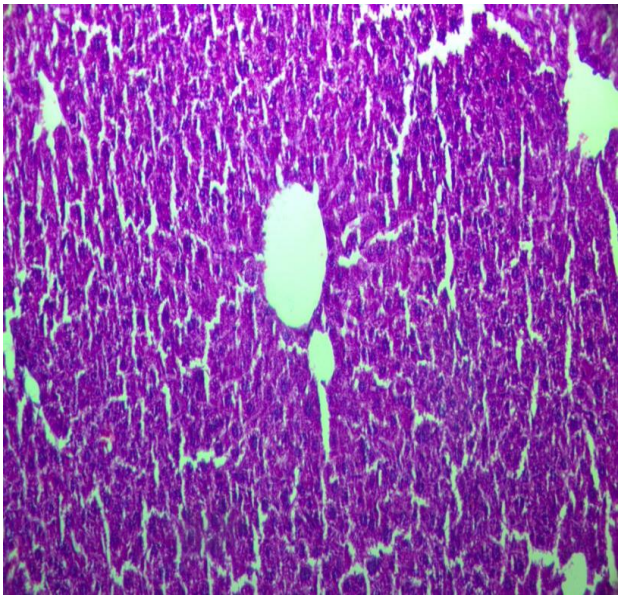

Figure 1: Section of liver from the control group showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100).

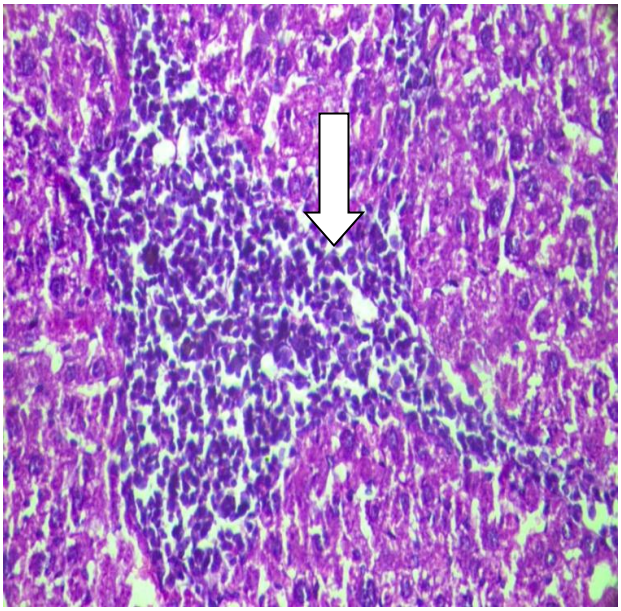

Figure 2: Liver section from mice treated with CCl_4 at a dose of 2 mg/kg; bw showing a focal mononuclear infiltration (arrow), H&E staining (x100).

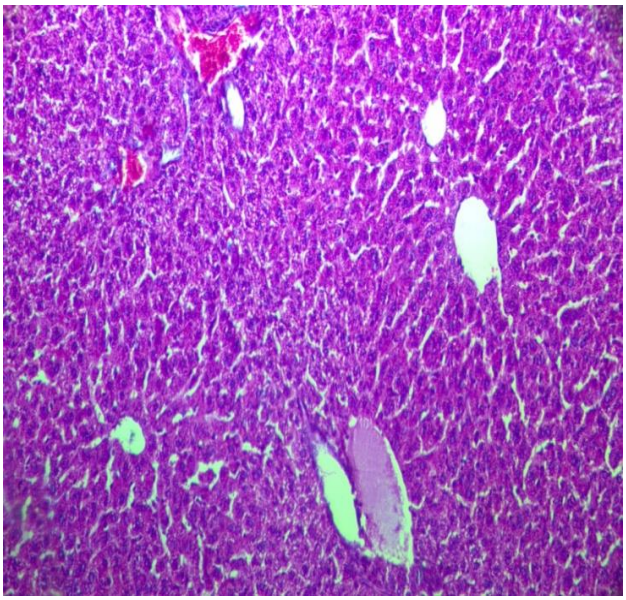

Figure 3: Liver section from mice treated with silymarin and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100).

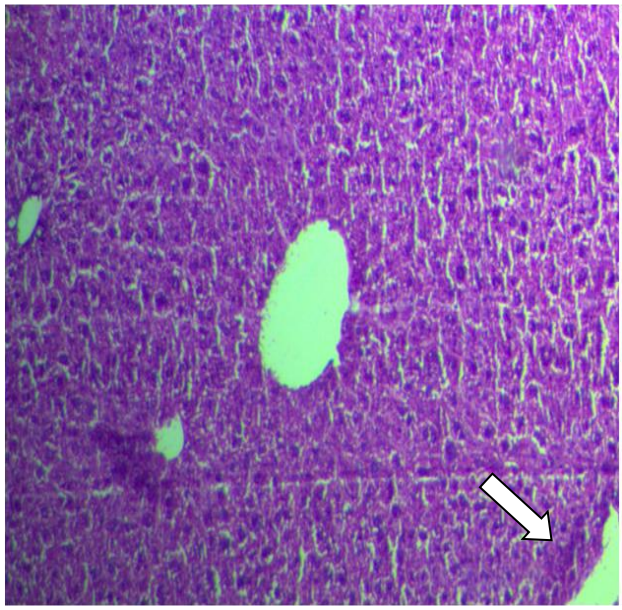

Figure 4: Liver section from mice treated with 200mg/kg *Lippia adoensis* aqueous extractandCCl_4 showing normal architecture of the hepatic lobule with minimal focus of mononuclear infiltration at the periphery (arrow) H&E staining (x100).

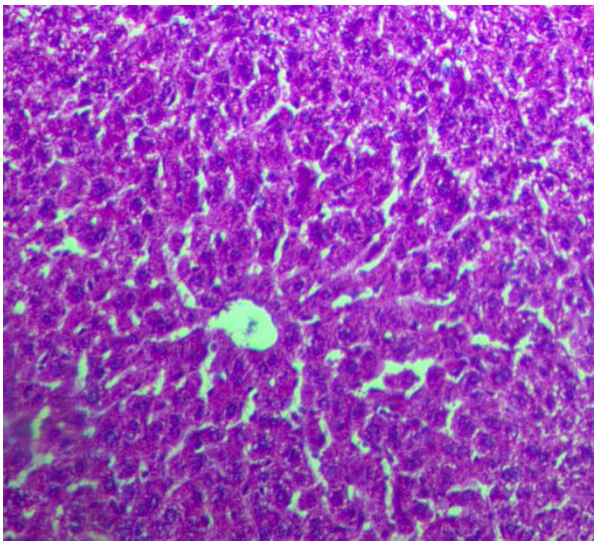

Figure 5: Liver section from mice treated with 400 mg/kg *Lippia adoensis* aqueous extract and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100).

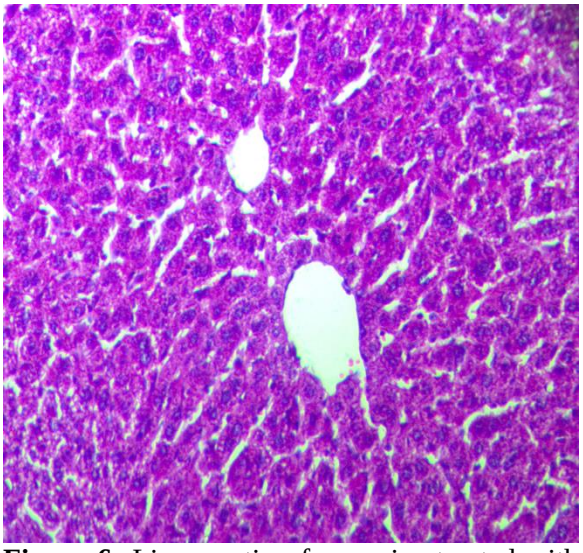

Figure 6: Liver section from mice treated with 200 mg/kg *Lippia adoensis* ethanolic extract and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H&E staining (x100).

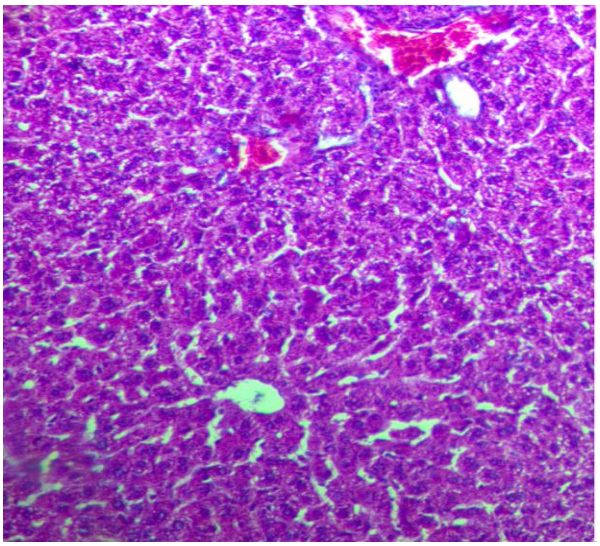

Figure 7: Liver section from mice treated with 400 mg/kg *Lippia adoensis* ethanolic extract and CCl_4showingnormal architecture of the hepatic lobule with no histopathological change H&E staining(x100).

5. Discussion

Medicinal plants are getting great attention as important sources of bioactive substances, with health beneficial effects. However, a great limitation to the use of medicinal plants is the issue of safety and toxicity. Liver damage is a widely used indicator of toxicity of medicinal plants *in vivo* (Kadejo *et al.,* 2012; Manuj *et al.,* 2014).

The aminotransferases (ALT, AST), ALP and bilirubin are among serum biomarkers of hepatic function, with their increase in the serum indicating hepatic damage (David *et al*., 2014), where as decreased level of total protein and albumin in the serum indicating hepatic damage (Kanwal *et al*., 2012).

Pretreatment of normal mice with *Lippia adoensis* did not result in any toxicity or adverse effects as indicated by the insignificant change in serum levels of ALT, AST, and ALP, total bilirubin, total protein and albumin in liver damage. Results from the present study confirmed that CCl_4 induced hepatotoxic effects manifested by a significant increase in activity of liver function marker enzymes ALT, AST, ALP and bilirubin in the serum of CCl_4 treated mice, whereas total protein and albumin in the serum of CCl_4 treated mice decreased significantly. These observations are in accordance with those in similar studies (Senthilkumar *et al.,* 2014; Isha *et al.,* 2014; Mary *et al.,* 2014).

Aminotransferases (ALT, AST) and ALP are cytoplasmic enzymes whose rise in serum levels are attributed to damaged structural integrity of the liver resulting from their released into the blood circulation after the rupture of the plasma membranes (Velmurugan *et al.,* 2014**;** David *et al.,*2013**).**

CCl_4 induced hepatic damage observed was confirmed by histopathological examination of the CCl_4 treated mice which revealed severe hepatic degeneration and marked foci of mononuclear infiltration of hepatic parenchyma tissue and around central vein, disorganization of hepatic plates and tissue necrosis (Snehal *et al*., 2014).

Lippia adoensis pretreatment of CCl_4 treated mice significantly reduced the elevated levels of ALT, AST, ALP and bilirubin. The diminished levels of these serum biomarkers may be attributed to the stabilizing effect of the *Lippia adoensis* phytochemical constituent(s) and various active ingredients on the plasma membrane of the hepatocytes, probably brought about by the stimulation of hepatocellular protein synthesis and ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of oxidative stress induced by CCl_4 (Farhan *et al*., 2012; Sagar *et al*., 2012).

Food rich in plant bioactive compounds and polyphenols and flavonoids in particular may exert beneficial effects towards human health (Francesco *et al.,* 2011). Health benefits of phytochemicals, especially antioxidant properties of phenolic compounds, which is known to exert preventive activity against degenerative diseases, inflammation and allergies via antioxidant, proteins and enzymes neutralization or modulation mechanisms (Tulay *et al*., 2014).

Among plants containing natural antioxidants, *Lippia adoensis* has attracted particular interest due to a high content of biologically active compounds (Riot *et al., 2005*). It has been considered to play an important dietary antioxidant role in the prevention of oxidative damage in living system (Tadewos *et al., 2014*).

The present study was done to demonstrate the hepatoprotective effect of aqueous and ethanolic extract of *Lippia adoensis* against carbontetrachloride induced liver damage. Carbontetrachloride is a widely used experimental hepatotoxicant biotransformed by cytochrome P-450 system to produce trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca^{2+} haemostasis and finally result in cell death (Mahmud *et al.,* 2012**).** Liver damage induced by carbontetrachloride is a classical model for screening the hepatoprotective activity (Showket *et al.,* 2014).

AST and ALT are found in serum and various body tissues but are mostly associated with liver parenchyma cells. Elevated levels of AST and ALT are observed in acute liver damage condition (Sakthivel *et al.,* 2012). In addition, the level of ALP rises with intrahepatic cholestasis and infiltrative diseases of the liver (Omar *et al.,* 2014).

The leakage of large quantities of enzymes into the bloodstream is associated with centrilobular necrosis of the liver **(**Hany *et al.,* 2013**).** Similarly in the present study, increases in serum enzyme level of ALT, AST, and ALP after exposing the mice to carbontetrachloride was observed confirming hepatic structural damage. In the present study, the levels of these enzymes were restored up to normal range after administration of *Lippia adoensis* extract indicating its hepatoprotective action. The reliable criterion for judging the quality of any hepatoprotective drug is to preserve the normal hepatic physiological functions that have been disturbed by hepatotoxins (Patrick *et al.,* 2012). Similar reports were observed from some other plant species including *Aerva lanata* (Kanchana *et al*., 2011), and *Red Lentil* (Rahmani *et al.,* 2013).

The serum levels of bilirubin, total protein and albumin were related to the function of hepatic cells (Hai *et al.,* 2014). A high concentration of bilirubin in serum is an indication for the cause of high erythrocytes degradation rate due to liver injury when treated with hepatotoxins (CCl_4) (Akram *et al.,* 2012). Reduction of total protein and albumin is also indication of liver damage resulting from defective protein biosynthesis in liver (Balaji *et al.,* 2013).

In the present study, the levels of bilirubin and total protein and albumin were restored to the normal values after administration of the plant extract indicating its hepatoprotective action. This effect was found to be comparable to that of standard drug (silymarin). The results generally suggest that the imbalanced antioxidant system in liver treated with carbontetrachloride is normalized by the protective effect of *L. adoensis* extract. The hepatoprotective effect of *L. adoensis* extract was further investigated by histopathological analysis.

Histopathological findings of liver samples were in agreement with the results obtained in serum biochemical studies, indicating that *L. adoensis* extract is able to protect carbontetrachloride induced hepatotoxicity. Phenolics and flavonoids display a wide range of biological and pharmacological properties, and they normally scavenge the free radicals and play an essential role in preventing oxidative stress (Kutaiba *et al.,* 2014).

It is well documented that *Lippia adoensis* is one of the rich sources of flavonoids, Phenolics, tannins, saponins and alkaloids *(*Riot *et al.,* 2005). Similar study was observed on the action of the crude flavonoids fraction of *Lippia nodiflora* (sudha *et al.*, 2013), and essential oil of *Lippia multiflora* in preventing oxidative stress (Kunle and Egharevba, 2012).

The outcome of the present investigation indicates that treatment with *L.adoensis* was effective in inhibiting the hepatotoxic effect of carbontetrachloride in vivo models, most likely because of high content of flavonoids, alkaloids, phenolics, tannins and saponins and active principle (Amita *et al.,* 2014). However, the precise molecular mechanism by which *L. adoensis* mediates its hepatoprotective action is still not clear.

In the present study, the normal liver tissue showed the typical architecture with a central vein and hepatocytes radiating from it. The portal triad consisted of hepatic artery, portal vein and bile duct which constituted various zones (1, 2 & 3) surrounding these areas. Carbontetrachloride treatment produced centrizonal necrosis (zone 3), marked foci of mononuclear infiltration to hepatic parenchyma tissue, sinusoid and around central vein, disorganization of hepatic plates (Gayathiri *et al.,* 2012).

Pretreatment with *Lippia adoensis* extract protected hepatic architecture and liver tissue from marked foci of mononuclear infiltration of hepatic parenchyma tissue, sinusoid and around central vein, as well as disorganization of hepatic plates and tissue necrosis, by preventing the toxic chemical reaction, oxidative stress, molecular changes in the liver tissues ultimately leading to necrosis. Similar reports were observed from some other plants including *veronica ciliatafisch* (Li *et al.,* 2014*), Mung Bean* (Norlaily *et al.,* 2013), *Deinococcus radiodurans* (Cheng *et al.,* 2014) and *Lumnitzera racemosa* (Sundaram and Murugesan, 2011).

Histopathological changes indicating liver damage after CCl_4 administration has been reported in the previous findings that CCl_4 causes necrosis (Sampath *et al*., 2013), fibrosis (Jamilah *et al.,* 2014),mononuclear cell infiltration (Jamilah *et al*., 2014),steatosis and degeneration of hepatocytes, in the liver and also CCl_4 causes apoptosis in liver (Sampath *et al.,* 2013). Therefore, histopathological findings in the liver due to CCl_4 administration are in agreement with previous studies.

Hepatoprotective drugs may play a role in the process of regeneration, prevention of fibrosis, or formation of nodules which may be expressed in the long term use of the drug (Elsohafy *et al.*, 2013). This study, however, showed marked foci of mononuclear infiltration to hepatic parenchyma tissue, sinusoid and around central vein, disorganization of hepatic plates and necrosis by hepatotoxic (CCl_4) and prevention of such changes and restoration to normal by *Lippia adoensis* extracts.

Histopathological examination of liver from CCl_4 intoxicated mice pretreated with *Lippia adoensis*, revealed enhanced hepatocellular architecture, which indicates the hepatoprotective effects of *Lippia adoensis*. The significant increase in body weight and decrease in absolute and relative liver weights in extract pretreated mice indicates that the plant extract protects liver from hepatotoxic agents. The results from the present study moreover reveal a relationship between body weight/absolute/relative liver weights and liver function.

Muhammad *et al*. and Douhri *et al.* reported similar results those of the present study. Bhaargavi *et al.* demonstrated that in hepatotoxicity, liver weight increases generally as a consequence of necrosis and fibrosis or hypertrophy of the liver and body weight decreases due to infiltration of hepatic parenchyma, disorganization of hepatic plates and fatty change. The alterations in the body weight and the liver weight in mice after CCl_4 administration were considered to result from direct toxicity of the liver and indirect toxicity related to liver damage (Andréia *et al.*, 2013). Change in relative liver weight is a valuable index of the extent of acute hepatic damage (Douhri *et al.*, 2014).

6. Conclusion

In the present study**,** it was proved that the aqueous and ethanolic extract of *Lippia adoensis* were found to be non toxic to mice up to the dose of 3000 mg/kg.p.o.

Aqueous and ethanolic extract of *Lippia adoensis* were effective in improving liver biochemical parameters and histopathological appearance of hepatocytes.

Aqueous and ethanolic *Lippia adoensis* extract exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity at the dose of 200mg/kg and 400mg/kg as shown from the investigated biochemical histological parameters. *Lippia adoensis* extracts were as effective as silymarin, which is a thoroughly researched plant in the treatment of liver diseases.

Lippia adoensis extracts were effective in maintaining the body weight changes and liver weight against carbon tetrachloride induced hepatotoxicity at the dose of 200mg/kg and 400mg/kg.

Therefore this study provides biological evidence supporting the use of *Lippia adoensis* as an adjuvant therapy for the prevention and treatment of liver disorders, however, a series of well controlled clinical intervention studies are needed to explore this possibility further more.

7. Recommendation

- The crude extracts of *Lippia adoensis* have shown potential hepatoprotective activity against carbon tetrachloride induced hepatotoxicity. Thus, further studies with purified fractions and bioactive compounds of *Lippia adoensis* and consequent hepatoprotective activity of the fractions is recommended.
- The active principles of *Lippia adoensis* responsible for the observed effect in the present studies should be identified more precisely
- The molecular mechanism of action needs to be explored
- Quantitative analysis should be done for each phytochemical constituent.
- The same work should be carried out to see other organs protective activities
- Similar work should be conducted on the same plant species collected from different locality in Ethiopia to see efficacy and potency of the plant in hepatoprotective effect.
- As it has been used as food condiment it is important to establish a range of values that can serve as a standard utilization of the plant species for its health benefit.

8. References

Abegaz, B., Asfaw, N. and Lwande, W. Constituents of essential oil from wild and cultivated *Lippia adoensis, J. Essent. Oil Res*.1993; 5:487-491.

Abhinav A, Mana R,Sarfaraz J,Ajay M,and Margaret E.Drug induced cholestatic jaundice.2014;3.http://dx.doi.org/10.1155/2014/260743.

Aggarwal BB, Sundram C, Prasad S, Reuter S, Kannappan R, Yadav VR, Park B, Kim JH, Gupta SC, Phromnoi K, Sung B. Chronic diseases caused by chronic inflammation require chronic treatment the anti-inflammatory life style. Inflammation, lifestyle and chronic diseases.2012; 373-446.

Akram Eidi, Pejman Mortazavi, Masoud Ebrahim Tehrani, Ali Haeri Rohani, Shahabaldin Safi.Hepatoprotective activity of cinnamon ethanolic extract against CCl_4-induced liver injury in rats .*EXCLI Journal* 2012; 11:495-507.

Al Meheithif A, Elnour A, Bamosa A and Aleissa K. Antioxidant effects of Zamzam water in normal rats and those under induced-oxidative stress. *Journal of Medicinal Plants Research* .2012; 6(42):5507-5512.

Alaezi CM, Adisa JO, Okorochi EC and Okechi OO. A progressive Nuclear stain substitute for haematoxylin And eosin staining technique in the Demonstration of invasive ductal Carcinoma. *GJSR Journal*.2014; 2(2):51-55.

Ali C, Guido G**.** Acute liver failure: a dangerous and challenging syndrome. *EMJ Hepatol*. 2014; 1:91-98.

Ambar K, Sandeep M, Atin K, Anoop S, Suresh CT. Endothelial function and oxidative stress in chronic kidney disease of varying severity and the effect of acute hemodialysis.2011; 33(4) 411-417.

Amita Jain, Rashmi Ranade, Prem Pritam, Neelu Joshi, Sirisha Lakshmi Vavilala, Ankita Jain. A comparative study of antioxidant activity, total phenolic and flavonoid contents in different parts of *Helicteres isora*. *L. American journal of life sciences*.2014; 2(5): 292-302.

Andréia Assunção Soares, Anacharis Babeto de Sá-Nakanishi, Adelar Bracht, Sandra Maria Gomes da Costa, Eloá Angélica Koehnlein, Cristina Giatti Marques de Souza and Rosane Marina Peralta. Hepatoprotective effects of *mushrooms*. Molecules.2013; 18:7609-7630.

Areefa,S. A Elumalai A, Chinna EM, Usha. Hepatoprotective medicinal plants. *Journal of Drug Delivery & Therapeutics.*2012; 2:2.

Asres K and Bucar F. Lippia adoensis var. Adoensis: studies on the essential oil composition and antioxidant activity, *Ethiop. Pharm. J.*2002; 20:32-38.

Asres, K, Bibbons, W, David, P. and Polo, M. Alkaloids of Ethiopian *Calpurniaaurea*, subsp. aurea, *Phytochemistry.*1986; 25(6): 1443-1447.

Avery, S. Molecular targets of oxidative stress. *Biochemical Journal*.2011; 434: 201-210.

Azza A, Naglaa Z and Kamel M. Protective effect of *Zingiber officinale* (ginger) on doxorubicin induced oxidative cardiotoxicity in rats. *J.Life Science.*2013; 10:2.

Balaji P, Kishore G and Yogendra V. In-vivo hepatoprotective activity of Barleria cristata L. ethanolic leaf extracts against CCl_4 induced hepatic injury in wistar rats. *Pharmacie Globale (IJCP).*2013; 4 (04).

Balamuruganvelu S, Geethavani B, Premlal K R, Sengottuvelu S, Jaikumar S. Hepatoprotective activity of polyalthia longifolia leaves against paracetamol induced hepatotoxicity in rats. *Sch. J. App. Med. Sci.*2014; 2(3A):908-910.

Bernard T. Guidelines on the care and use of animals for scientific purposes. *National advisory committee for laboratory animal research (NACLAR).* 2004; 15-18.

Bhaargavi, Vuyyuri, G. S. L. Jyotsna, and Reshma Tripurana. "A review on hepatoprotective activity." *International Journal of Pharmaceutical Sciences & Research*. 2014; 5(3): 690-702.

Bhaskara KL and Mukalel SL. Curative efficacy of methanolic extract of Monochoria vaginalis against Carbontetra chloride (CCl4) induced liver fibrosis. *Journal of Pharmacy and Biological Sciences (IOSR-JPBS).* 2014;9(1) :71-76.

Chao J, Liao J-W, Peng W-H, Lee M-S, Pao L-H, Cheng H-Y. Antioxidant, Analgesic, Anti-Inflammatory, and Hepatoprotective Effects of the Ethanol Extract of *Mahonia oiwakensis* Stem. *International Journal of Molecular Sciences*.2013; 14(2):2928-2945.

Chen C.J., Deng A.J., Liu C, Shi R, Qin HL, Wang AP. Hepatoprotective activity of Cichorium endivia L. extract and its chemical constituents. *Molecules.2011;16:9049-9066.*

Cheng, Jianhui, Zuofa Zhang, Zhiguo Zheng, Guoying Lv, Liangyan Wang, Bing Tian, and Yuejin Hua. "Antioxidative and Hepatoprotective Activities of Deinoxanthin-Rich Extract from *Deinococcus radiodurans* R1 against Carbon Tetrachloride-Induced Liver Injury in Mice." *Tropical Journal of Pharmaceutical Research* 2014; 13(4):581-586.

Comfort JA, Augusta AE, Gbadebo EA, Margaret OO and Emmanuel NM. N-Nitrosation of dimethylamine hydrochloride and its toxicology in the wistar rats fed different levels of dietary protein. *European Journal of Experimental Biology.* 2013; 3(2):94-103.

David A. Areshidze, Lyudmila D. Timchenko, Maria A. Kozlova. Protective effect of enzymatic hydrolyzate of *Chlorophytum comosum* (L.) against experimental toxic liver injury in Wistar rats at the age of two years. *American Journal of Bioscience and Bioengineering* .2013; 1(4): 49-54.

David EK, Naga PC, William ML. Hepatic histological findings in suspecteddrug-induced liver injury. Hepatology.2014; 59(2): 661–670. doi:10.1002/hep.26709.

Davies KJA.Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. *IUBMB Life.*2000;50: 279-289.

Douhri B.,Idaomar M, Skalisenhaj N, Ennabili A, Abrini J. Hepatoprotective effect of *Origanum elongatum*. *European journal of medicinal plants 2014;*4(1): 14-28.

Du J, Cullen J.J, Buettner GR. Ascorbic acid: chemistry, biology and the treatment of cancer. Biochimica et Biophysica Acta.2012;1826: 443-457.

Dumas B, Watson W, Biggs M. Albumin standard and the measurement of serum albumin with bromocresol green. Cli.Chem. Acta., 1971 (31): 87-96.

El Sohafy, Samah M., Saleh I. Alqasoumi, Aly M. Metwally, Abdallah A. Omar, Masouda M. Amer, Mohammed I. Abou Shoer, S. A. El Toumy, and Maged S. Abdel-Kader. "Evaluation of the hepatoprotective activity of some plants belonging to the tribe Cynareae growing in Egypt." *Journal of Medicinal Plants Research.* 2013; 7(7):324-328.

Eman A,Olli M, Sayed HS, Abdel NA.Hepatoprotective and antioxidant effect of *Bauhinia hookeri* extract against carbon tetrachloride-induced hepatotoxicity. *BioMed Research International*. 2014; 9. http://dx.doi.org/10.1155/2014/245171.

Farhan H, Rammal H, Hijazi A,Hamad H, Daher A, Reda M, Badran B. In vitro antioxidant activity of ethanolic and aqueous extracts from crude *malva parviflora*. Grown in lebanon. *Asian J Pharm Clin Res*.2012; 5(3):234-238.

Francesco Visioli,Catalina Alarcón,Cristina Andres, Michael Aviram, Conceição Calhau,Alfredo Cassano, Massimo D'Archivio, Ana Faria,Gaëlle Favé,Vincenzo Fogliano,Rafael Llorach. Polyphenols and human health. *Critical Reviews in Food Science and Nutrition*.2011; 51:524–546.

Gayathiri S, Vetriselvan S, Shankar J, Ishwin S, Hemah D, Shereenjeet K,Yaashini A. Hepatoprotective activity of aqueous extract of *hippophae rhamnoides.* International *journal of biological & pharmaceutical research.* 2012; 3(4): 531-537.

Gilani AH, Janbazz KH. Preventive and curative effects of Ber-beris aristata fruit extract on paracetamol and CCl_4-induced hepatotoxicity. *Gen Pharmacol* 1995; 26:627-31.

Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B & Pradhan SC. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. *Indian J Med Res.*2009; 129:569-578.

Gornall A, Charles J, Maxima M. Determination of serum proteins by means of the biuret reaction, *J. Biol. Chem.,* 1949 (177): 751–766.

Hai Zhong Huo, Bing Wang, Yong Kang Liang, Yong Yang Bao and Yan Gu. Hepatoprotective and antioxidant effects of licorice extract against ccl4-induced oxidative damage in rats. *Int. J. Mol. Sci.* 2011;12: 6529-6543.

Halina CL and Agata M. Oxidative stress as a crucial factor in liver diseases *World J Gastroenterol*.2014; 20(25): 8082–8091.

Halliwell B, Gutteridge J.M.C. Free radicals in biology and medicine. Fourth edition. Clarendon Press, Oxford UK. 2007.

Hany M. Yehia, Ebtesam M. Al-Olayan,Manal F. Elkhadragy. Hepatoprotective role of the Pomegranate (Punica Granatum) Juice on Carbon Tetrachloride-Induced Oxidative Stress in Rats. *Life Sci J*. 2013; 10(4): 1534-1544.

Hashemy SI. The human thioredoxin system: modifications and clinical applications. Iranian *Journal of Basic Medical Sciences.*2011;14:191-204.

Imran Q,Muhammad A, Muhammad A, Mohammad S and Muhammad H. Hepatoprotective activity of aqueous methanolic extract of Viola odorata against paracetamol induced liver injury. *Bangladesh J Pharmacol.* 2014; 9: 198-202.

Isha I. Sait, Jyoti Harindran, A. Abdul Vahab, Jeena J. L, Nasli S. Jolly John. Potential hepatoprotective effect and antioxidant role of methanol extract of morinda tinctoria in carbon tetrachloride induced hepatotoxicity in albino rats. *Int J Pharm* 2014; 4(1): 363-368.

Iyanda A A and Adeniyi F A. Buffer conditions for measuring alkaline phosphatase activity in Indices of Hepatorenal Function. *J Phys Pharm Adv*. 2014; 4(1): 310-317.

Jamilah M Hashemi. Hibiscus Sabdariffa Calyx Extract alleviate hepatotoxicity induced by carbon tetrachloride on male albino rats. *Nat Sci.*2014;12(6):111-120.

Jayashree BC, Fiona O, and Meagan J. Walsh. Mechanisms and biomarkers of apoptosis in liver disease and fibrosis, *International Journal of Hepatology* 2012; 10:10.

Joubert E, Winterton P, Britz T J, Gelderblom WCA. Antioxidant and pro-oxidant activities of aqueous extracts and crude polyphenolic fractions of rooibos (Aspalathus linearis). *Journal of Agricultural and Food Chemistry*.2005; 53: 10260-10267.

Kadejo OA, James DB, Sallau BA, Ozigi A A . In vivo antioxidant effect of aqueous root bark, stem bark and leaves extracts of Vitex doniana in CCl_4 induced liver damage rats. *Asian Pac J Trop Biomed*. 2013; 3(5): 395-400.

Kanchana N, and Sadiq MA. Hepatoprotective effect of Plumbago zeylanica on paracetamol induced liver toxicity in rat. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2011; 3(1):151–154.

Kang H and Koppula S. Hepatoprotective effect of *Houttuynia cordata* thunb extract against carbon tetrachloride-induced hepatic damage in mice. *Indian J Pharm Sci.* 2014 ;76(4): 267–273.

Kanwal Z, Muhammad IS, Muhammad AM. Effect of *Momordica charantia* (bitter melon) on serum glucose level and various protein parameters in acetaminophen intoxicated rabbits.*J Intercult Ethnopharmacol*.2012; 1(1):7-12.

Klaunig J.E, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicologic Pathology.2010; 38: 96-109.

Kumari R, Prakash R, Suman P K. and Padmadeo S R. Hepatoprotective activity of azadirachta indica leaves. *International journal of basic & applied science research*.2014;1(1): 28-34.

Kundu JK, Surh YJ. Emerging avenues linking inflammation and cancer. Free Radical Biology and Medicine.2012; 52: 2013-2037.

Kunle OF and Egharevba H O . Essential oil of *Lippia multiflora moldenke .Journal of applied pharmaceutical science*.2012; 02 (01): 15-23.

Kutaiba Ibrahim Alzand, Mohamed Ali Abdullah, Mukul Tailang. Structural elucidation and biological activity of the flavonoid from the roots of cudrania tricuspidata. *World Journal of Pharmaceutical Research*. 2014; 3(10): 981-997.

Lambert J.D, Elias RJ. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Archives of biochemistry and biophysics. 2010;501:65-72.

Lambert J.D, Sang S, Yang CS. Possible controversy over dietary polyphenols: benefits vs risks. *Chemical Research in Toxicology*.2007; 20: 583-585.

Li Yin , Lei Wei , Rao Fu , Lisheng Ding , Yiran Guo , Lin Tang and Fang Chen. Antioxidant and hepatoprotective activity of *veronica ciliata fisch* Extracts against carbon tetrachloride-induced liver injury in mice. Molecules.2014; 19:7223-7236.

Luis R, Martínez-Castelao D, Górriz A, De-Álvaro JL, Navarro-González F. Pathophysiological role and therapeutic implications of inflammation in diabetic nephropathy. *World Journal of Diabetes.* 2012; 3: 7-18.

Magda KE, Germine MH and Rasha MA. The Synergistic Hepatoprotective Effect of *Curcumina* and *Ginger* against carbon tetrachloride induced- liver fibrosis in Rats. *Journal of Basic and Applied Sciences* 2011; 5(9): 1962-1971.

Maliha AAM. Phenoli. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transminases. *Journal of Food and Nutrition Sciences.*2014; 2(5):207-214.

Manuj Kr. Bharali, Hage Konya, Luk Bahadur Chetry. Protective effect of *Oroxylum indicum* on acetaminophen induced liver injury in rat. *International Current Pharmaceutical Journal, January* 2014, 3(2): 223-227.

Mary M, Jennifer J, Leonid K.Toxicological review of carbon tetrachloride.2010; 56-23-5.

Mary Tolulope Olaleye, Ayodeji Emmannuel Amobonye, Kayode Komolafe ,Afolabi Clement Akinmoladun. Protective effects of *Parinari curatellifolia* flavonoids against acetaminophen-induced hepatic necrosis in rats. *Saudi Journal of Biological Sciences.*2014; 21:486–492.

Mehwish JA and Syeda NF. Prevention of liver cirrhosis by green tea supplementation. *World J Pharm Sci*. 2014; 2(8): 716-724.

Michael PH and ynthia Ju. Mechanisms of drug-induced liver injury. *The AAPS Journal* .2006; 8 (1): E48–E54.

Michael C K. Hepatocellular carcinoma.*Journal of Hepatocellular Carcinoma*. 2014; 1:115–125.

Michael DL,John JP, and Jayant AT. Drug-induced liver injury. Mayo Clin Proc. N. 2014;89(1):95-106.

Mohamed STS, Madhusudhana C, Chetty S, Ramkanth V.S.T, Rajan KM.Hepatoprotective herbs. *Int. J. Res. Pharm* Sci. 2010;1:1-5.

Mohamed,Z.M., Salem,JO, Salem A.Z.M. biological activities and phytochemicals composition of Hibiscus species. *Life Science Journal.* 2014;11(5): 1097-8135.

Montuschi P, Barnes PJ, Roberts L.J. Isoprostanes: markers and mediators of oxidative stress. *FASEB Journal.* 2004;18: 1791-1800.

Muhammad Imran, Muhammad Ali, Muhammad Ali, Mohammad Saleem and Muhammad Hanif. Hepatoprotective effect of leaves of aqueous ethanol extract of *Cestrum nocturnum*. *Bangladesh J Pharmacol* 2014; 9:167-170.

Muhammad k, Ahmad ZS, Tahira B, Abdurahman H. In vitro anti-acetyl cholinesterase and antioxidant activity of selected Malaysian plants. *Asian J Pharm Clin Res*.2014;7(3):93-97.

Nadro MS ,Onoagbe IO.Protective effects of aqueous and ethanolic extracts of the leaf of *Cassia italica* in CCl_4-induced liver damag in rats. *American Journal of Research Communication.*2014;2(6): 122-130.

Nancy VM, Eduardo MS, Ángel MG, Jaime ES, Cesar EC, Manuel LG, Juan AG, and José AM. Hepatoprotective effect of silymarin. *World J Hepatology*. 2014; 6(3): 144–149.

Nordberg,J,Arnér, ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free radical biology and medicine*.2001; 31: 1287-1312.

NorlailyMohd Ali,HamidahMohd Yusof,Kamariah Long,Swee Keong Yeap,Wan Yong Ho. Antioxidant and Hepatoprotective Effect of Aqueous Extract of *Germinated* and *Fermented Mung* Bean on Ethanol-Mediated Liver Damage. *Bio Med Research International*. 2013; 9.

Omar Rashid Sadeq., Ismail AL-Masri and Khalil AL-Kharaz. The effect of ibuprofen on hepatic glutamic pyruvictransaminase (sgpt), glutamic oxaloacetic transaminase (sgot) and alkaline phosphatase (alp) in dental patients. *Int J Pharm Bio Sci* 2014 Oct; 5(4): (P) 689-691.

Pamplona R, Costantini D. Molecular and structural antioxidant defenses against oxidative stress in animals. *American Journal of Physiology*.2011; 301: R843-R863.

Pandey K.B, Rizvi S.Resveratrol may protect plasma proteins from oxidation under conditions of oxidative stress in vitro. *Journal of the Brazilian Chemical Society*.2010; 21: 909-913.

Patrick IK, Amadi U, Charles IA, Ayalogu EO. Evaluation of acute and sub-chronic oral toxicity study of baker cleansers bitters a polyherbal drug on experimental rats. *Excli Journal*.2012; 11:632-640.

Pitocco D,Zaccardi F, Di S,Romitelli E , Santini F, Zuppi S.A, Ghirlanda C. Oxidative stress, nitric oxide, and diabetes. *The Review of Diabetic Studies*.2010;7: 15-25.

Punitha SC and Rajasekaran M. Antioxidant mediated defense role of *Wedelia calendulacea* herbal extract against CCl4 induced toxic hepatitis. *Journal of Applied Pharmaceutical Science.2011;* 01 (09): 111-115.

Rahmani AH, Goudarzi M, Rashidi MR , Houshmand Gh , Khadem HH. Protective Effect of *Red Lentil* (*Lens Culinaris*) Extract against Carbon Tetrachloride-Induced Hepatotoxicity in Mice. *J Babol Univ Med Sci*.2014; 16(2): 49-55.

Rajeev k, Manish G, Sanjeev M. Hepatotoxicity and Functional anatomy of the liver. *Journal of scientific & innovative research*. 2012; 1 (1): 2012 :43-54.

Ramadan S I., Shalaby MA, Nehal A and El-Banna HA.. Hepatoprotective and Antioxidant Effects of Silybum marianum Plant in Rats .*IJAVMS.*2011;5(6): 541-547.

Raúl JA, Mercedes R, Alejandra FC, Susana LO, Carmen LV, Isabel ML. Assessment of drug-induced hepatotoxicity in clinical practice.A challenge for gastroenterologist.*World J Gastroenterol*.2007; 13(3): 329-340.

Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanism of carbon tetrachloride toxicity. *Pharmacol Ther.* 1989;43:139-54.

Reitman S, Frankel SA.Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Path*., 1957 (28):56-63.

Richard JY, Andreas S. The BCL-2 protein family: opposing activities that mediate cell death. Molecular Cell Biology.2008; 9:47-59. | doi:10.1038/nrm

Riot Sishu, Mariamawit Yonathan, Ameha Seyoum and Kaleab Asres. Radical scavenging activity of volatile oils of herbs traditionally used to spice cooking butter in Ethiopia. *Ethiopian Pharmaceutical Journal.*2005;23:7-14.

Robert J. Fontana RF. Schwabe and John WW. Pathogenesis of idiosyncratic drug-induced liver injury. Gastroenterology .2014;146:914–928.

Russel JR, Dun-xian T and Annia G .Melatonin reduces lipid peroxidation and membrane viscosity front. *Physiol.* 2014; 5:377.

Sabreena Rashid, Zahoor A Kaloo, Seema Singh & Iram Bashir. Medicinal importance of Genus Rheum on hepatoprotective, *International Journal of Advanced Research*. 2014; 2(7):261-267.

Sagar D, Varsha Z, Dinesh D, Shital P. Hepatoprotective activity of Alcoholic leafextract of *Alianthus exelssa* root against carbon tetrachloride toxicity induced in albino rat. *Int.J. PharmTech Res.*2012; 4(2).

Sakihama Y,Cohen M.F, Grace S.C,Yamasaki H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology.2002; 177: 67-80.

Sakthivel KM, Kannan N, Angeline A, Guruvayoorappan C. Anticancer Activity of *Acacia nilotica* (L.) Wild. Ex. Delile ubsp. indica against dalton's ascitic lymphoma induced solid and ascitic tumor model. *Asian Paciic J Cancer Prev*.2012; 13:3989-3995.

Saleem TSM, Hetty,C.M.,Ramkanth,S.,Rajan,V., Kumar,K.M., Gauthaman,K. Hepatoprotective herbs-a review. *International Journal of Research in Pharmaceutical Sciences*.2010;1:1-5.

Sampath Kumar GV, Sri Vidya VVL, Ch.Sandhya. Hepatoprotective activity of *borreria hispida l*.Roots against carbon tetrachloride induced hepatotoxicity in albino wistar rats. *International Journal of Biological & Pharmaceutical Research.* 2013; 4(12): 1277-1282.

Sanjay A, Shravan KG, Paresh L, Pravin Y, Mangesh L. Hepatoprotective activity of herbal formulation against paracetamol induced hepatotoxicity. *Indo American Journal of Pharmaceutical Research.*2014, ISSN NO: 2231-6876.

Sarah O. Nwozo, Adeyinka A. Ajagbe, Babatunji E. Oyinloye. Hepatoprotective effect of Piper guineense aqueous extract against ethanol-induced toxicity.*Journal of Experimental and Integrative Medicine.* 2012; 2(1):71-76.

Seifert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schröter F, Prozorovsky, T, Lange N, Steffen J, Rieger M. Immuno proteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell.2010; 142: 613-624.

Sekar Suresh, Anandan Balakrishnan, Raziya Banu, Seppan Prakash.Ageing Induced Changes in Ventricular Myocardium. A histological and histomophometrical study.*International Journal of Anatomical Sciences, 2013, 4(2): 03-10.*

Senthilkumar,Ravichandran, Rahul Chandran, and Thangaraj Parimelazhagan. "Hepatoprotective effect of *Rhodiola imbricata* rhizome against paracetamol-induced liver toxicity in rats." *Saudi journal of biological sciences.*2014; 21(5): 409-416.

Sharma Bhawna, Sharma Upendra Kumar.Hepatoprotective activity of some indigenous plants. *International journal of pharmtech research*. 2010; 2(1):568-572.

Showket Hussain Bhat,Rajni Shrivastava, Mohd Yousuf Malla, Mohd Iqbal, Tahira Anjum, Abrar Hussain Mir, Manik Sharma. Hepatoprotective activity of *argemone mexicana* linn against toxic effects of carbon tetrachloride in rats. *World Journal of Pharmaceutical Research*.2014;3 (3) :4037-4048.

Singh,A,BhatTK, Sharma OP. Clinical Biochemistry of hepatotoxicity.*J Clinic Toxicology.*2011; S4:001. doi:10.4172/2161-0495.S4-001.

Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC. Oxidative stress, anti-oxidant therapies and chronic kidney disease. Nephrology. 2012;17: 311-321.

Snehal Gite, Supriya Yadav, Smita Nilegaonkar, Vaishali Agte. Evaluation of hepatoprotective potential of functional food formulations using in vitro and in vivo models of CCl_4 radical induced toxicity. *International Journal of Interdisciplinary and Multidisciplinary Studies (IJIMS),* 2014:1(10) 6-13.

Street R A and Prinsloo G. commercially important medicinal plants of south africa, *Journal of Chemistry.*2013; 10:6.

Sudha A, Sumathi K, Manikandaselvi S, Prabhu NM and Srinivasan P.Anti-hepatotoxic Activity of Crude Flavonoid Fraction of *Lippia nodiflora* on ethanol induced liver injury in rats**,** *Asian Journal of animal Sciences.*2013; 7(1):1-13.

Sundaram R, Murugesan G. Hepatoprotective and antioxidant activity of a mangrove plant *Lumnitzera racemosa. Asian Pac J Trop Biomed. 2011; 1(5): 348-352.*

Sunita Shailajan, Mayuresh Joshi, Bhavesh Tiwari.Hepatoprotective activity of Parmelia perlata (Huds.) Ach. against CCl4 induced liver toxicity.*Journal of Applied Pharmaceutical Science.* 2014; 4 (02):070-074.

Tadewos H, Abadi G, Henok K. Evaluation of physico-chemical, microbial and organoleptic properties of cow milk in smoked container with olive (*Olea africana*) and added leaves of *Lippia adoensis*. *American Journal of Food Science and Nutrition*.2014; 1(2): 29-37.

Tang,S.Y.,Halliwell,B. Medicinal plants and antioxidants, from cell culture and Caenorhabditiselegans studies.*Biochemical and BiophysicalResearchCommunications.*2010;394: 1-5.

Toshihiro,A., Gebreyesus, T., Hiroshi, H. and Toshitake, T. Co-occurrence of C- 24 Epimeric 24-ethylsterols Possessing and Lacking a 25 bond in Kalanchoe petitiana, Phytochemistry.1992; 31: 163-166.

Tulay Ozcan,Akpinar BA, Yilmaz EL, and Delikanli B. Phenolics in Human Health. *International Journal of Chemical Engineering and Applications*.2014;5(5):393-396.

Valko M, Rhodes C,Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions.2006;160:1-40.

Velmurugan V, Arunachalam G. Hepatoprotective activity of methanol extract of stem bark of *prosopis cineraria linn* against carbon tetrachloride induced hepatotoxicity. *Int J Pharm Pharm Sci*.2014; 6(2) :491-493.

Young D, Pestaner L, Gibberman P. Effects of drugs on clinical laboratory tests. Clin Chem. 1975;(21): 246-248.

Zhang Y, Yang ND, Zhou F, Shen T, Duan T, Zhou J, Shi Y, Zhu XQ, Shen HM.(−)-Epigallocatechin-3-gallate induces non-apoptotic cell death in human cancer cells via ros-mediated lysosomal membrane permeabilization. *PLoS ONE*.2012; 7: e 46749.