THE ROLE OF CRUDE ETHANOL EXTRACT OF GINGER RHIZOME (*Zingiber officinale*) ON THE LIVER BIOMARKERS OF CCl_4-INDUCED HEPATOTOXICITY IN MALE WISTAR ALBINO RATS.

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"Words are never enough when it comes to state what goes on beyond the soul." Just like now…

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List of Acronyms

CCl_4	Carbon tetrachloride
iNOS	Induced nitric oxide
COX-2	cyclooxigenase-2
SOD	Superoxide dismutase
IκBα	Inhibitor kappa B alpha
AP-1	Activated protein-1
TNF-α	Tumor necrosis factor alpha
NF-κB	Necrosis factor kappa B
ROS	Reactive oxygen species
MDA	Malondialdehyde
GPx	Glutathione peroxidase
LTA4H	Leukotriene A4 Hydroxylase
MAPK	Mitogen activator protein kinase
ERK2	Extracellular regulatory kinase-2
CRE	Cyclic AMP Response element
PI3K	Phosphoinositide-3 kinase
GST	Glutathione-s-transferase

Cyp2E1	Cytochrome p450 isozyme
H2O2	Hydrogen peroxide
HDL-C	High Density Lipoprotein-Cholesterol
LDL-C	Low Density Lipoprotein-Cholesterol
TC	Total cholesterol
TG	Triglycerides
TP	Total proteins
DB	Direct bilirubin
TB	Total bilirubin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
GRAS	Generally Recognized as Safe
ELISA	Enzyme Linked Immune Sorbent Assay

ABSTRACT

Introduction: Liver, an imperative organ has a crucial role in the metabolism of xenobiotics that causes it to succumb to numerous hepatic diseases. Synthetic drugs exploited in the treatment of liver diseases are incompetent and may sometimes lead to serious side-effects. In this context, herbal therapy has emerged as a proficient approach with good values in treating hepatic diseases. Ginger have been proven to be effectual as hepatoprotective agent and it may serve as a vital source of potentially useful new compounds for the development of effective therapy to combat a variety of liver problems caused by many toxins including carbon tetrachloride.

Objectives: The present study was designed to explore the role of crude ethanol extract of ginger rhizome on the biomarkers of CCl_4 induced hepatotoxicity in male Wistar albino rats.

Methods: Twenty eight rats were used throughout the study. Rats were divided into four groups with seven in each. Group 1 (normal control) received 1mL/kg of corn oil, group 2 (standards) received 350mg/kg vitamin E, group 3 (treated) received 200mg/kg crud ginger extract, group 4 (negative control) received 0.5mL/kg CCl_4. Liver toxicity was induced by intraperitoneal administration of CCl_4 dissolved in 1mL corn oil at a dose of 0.5mL /Kg body weight.

Administration of crude ethanol ginger extract at a dose of 200 mg/kg body weight was started a week after CCl_4 treatment and was performed for five weeks (totally six weeks).

Results: The results revealed that hepatotoxic rats supplemented with 200mg/kg showed a significant decrease in MDA, ALT and TP with the same p-value ($p<0.01$) and AST ($p<0.001$) as compared to negative control. ALP ($p<0.01$), DB ($p<0.05$) and TB ($p<0.001$) in treated groups compared to the negative control, but no significant difference was observed in treated, normal and the standards. Serum TC, LDL-c, and TG were increased significantly ($P<0.05$) in CCl_4 treated group, while HDL-c level was decreased significantly compared to the rest of the groups. In ginger treated group, the serum levels of TC, LDL-c, and TG were significantly ($p<0.001$) decreased, but HDL-c was significantly ($p<0.05$) higher than negative control. Serum levels of TC, LDL-c, and TG in standards were decreased significantly ($P<0.001$), but HDL-c increased none significantly compared to negative control. The levels of TC, LDL-c, HDL-c, and TG in treated group were non-significantly different compared to the normal control and the standards.

Conclusion: The study revealed that ginger rhizome showed a promising hepatoprotective effect, because it contains antioxidants and potent lipid lowering phytoconstituents. Despite the above, further study is recommended to investigate the active ingredients of the extract and their exact mechanism of action.

Key points: *Zingiber officinale*; hepatoprotective; antioxidant; carbon tetrachloride, MDA, Liver function enzymes, lipid profiles.

1. INTRODUCTION

1.1. Metabolic Functions of the Liver

Liver is a large, bilobed, and complex organ receiving a large amount of blood and nutrients from the gastrointestinal system. It also contains Kupffer cells as well as tissue lymphocytes and fibroblasts, key contributors in immune defenses (Mutter *et al*., 2010).
The liver is composed of a variety of microscopic functional units, called lobules. Lobules are comprised chiefly of sheets of hepatic parenchymal cells or hepatocytes which are chemically reactant pool of cells that have a high rate of metabolism, sharing substrates and energy from one metabolic system to another, processing and synthesizing multiple substances that are transported to other areas of the body, and performing myriad of other metabolic functions like secretion and storage and is the frequent target for a number of toxicants (Meyer and Kulkarni, 2001).

Liver has a variety of key functions, including metabolism of the products of ingested food, production of amino acids to form proteins, detoxification of ingested drugs, conversion of nitrogenous substances from the gut into urea for subsequent renal excretion, formation of clotting factors, metabolism of bilirubin, processing of lipids absorbed from the intestine, and excretion of its products as bile (Sekiwa *et al*., 2000).These various functions are achieved by several types of liver cells, including hepatocytes, bile duct cells, and Kupffer cells.

The liver has a dual blood supply that is 70% of its blood is delivered by the portal vein, which drains the intestine, while the remainder is from the hepatic artery (Goldman and Andrew, 2008). After delivery to the liver by the portal vein, nutrient-rich blood passes along the hepatic sinusoids in close contact with the hepatocytes that line them before draining into the hepatic vein. The hepatocytes detoxify, metabolize, and synthesize the products of digestion delivered to them. Clinical consequences of liver disease may reflect the loss of hepatocellular activity, with diminished detoxification, excretory, and synthetic functions and interruption of bile flow may be caused by ineffective biliary excretion of diseased hepatocytes.

Hepatocyte dysfunction results in diminished production of clotting factors, albumin, and other proteins, as well as the reduced endogenous formation of lipids and reduced removal of lipoproteins from the body (Mutter *et al*., 2010).Hepatocyte injury from a variety of causes, including viruses, alcohol, autoimmune disorders, and drug hepatotoxicity is accompanied by leakage of cellular enzymes into the systemic circulation.

Coagulopathy, decreased serum albumin, and hyperbilirubinemia are typical in more profound hepatocellular injury. Therefore, damage on the liver inflicted by hepatotoxic agents is of grave consequences (Shanmugasundaram and Venkataraman, 2006).

1.2. Bilirubin Metabolism in the liver

Bilirubin, the product of the breakdown of red cells and other hemoproteins (a class of proteins involved in the transport or metabolism of oxygen), is produced by reticuloendothelial cells predominantly in the liver and spleen. Bilirubin is transported to the hepatocytes while bound to albumin; it is then solubilized by the hepatocytes for excretion into the bile ducts (Goldman and Andrew, 2008).

Normal adults produce about 4 mg of bilirubin per kilogram of body weight per day. Between 70 and 90% of bilirubin is derived from the hemoglobin of erythrocytes, which in turn are sequestered and destroyed by the mononuclear phagocytic cells of the reticuloendothelial system, principally in the spleen, liver, and bone marrow a minor fraction reflects ineffective erythropoiesis, the premature destruction of newly formed erythrocytes within the bone marrow (Goldman and Andrew, 2008).The remainder results primarily from the turnover of non hemoglobin hemoproteins such as myoglobin, the P-450 cytochromes, catalase, and peroxidase. Although this occurs principally in the liver, bilirubin has proved to have antioxidant properties, and recent studies suggest that a limited, regulated production of bilirubin from heme may occur in many cell types and contribute to regulating the intracellular antioxidant environment.

The two-step conversion of heme to bilirubin commences with the opening of the heme molecule at its α bridge carbon by the microsomal enzyme *heme oxygenase*, a process that results in the formation of equimolar quantities of carbon monoxide and the green tetrapyrrole biliverdin, the main excretory product of heme in birds, reptiles, and amphibians (Goldman and Andrew, 2008). Because biliverdin cannot cross the placenta, its reduction to bilirubin in mammals by a second enzyme, *biliverdin reductase*, allows its transplacental removal from the fetus into the maternal circulation.

The unconjugated bilirubin (UCB) produced in the periphery is transported to the liver in plasma (**Figure 1.1**) Because of its insolubility in aqueous media; it is kept in solution by tight but reversible binding to albumin.

A number of compounds, including sulfonamides, furosemide, and radiographic contrast agents, competitively displace bilirubin from its binding sites on albumin, a phenomenon of little clinical significance except in neonates, in whom the resulting increased concentration of unbound bilirubin raises the risk of kernicterus.

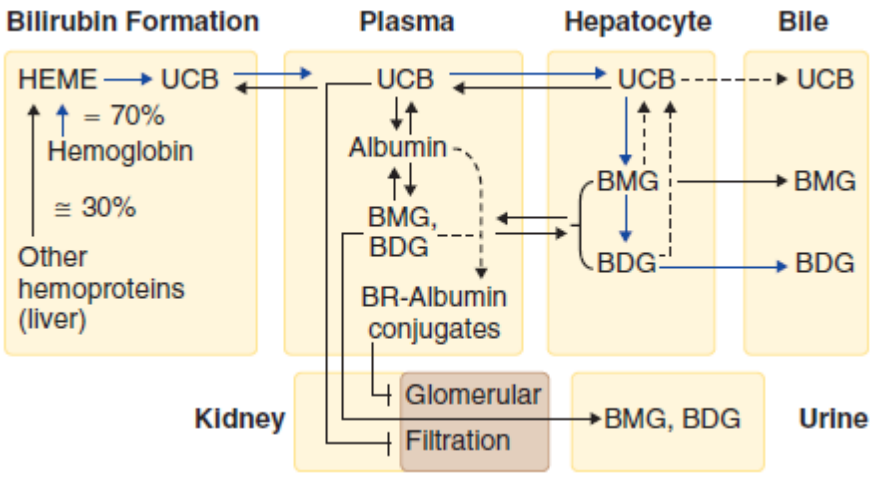


Figure 1.1 *Overview of bilirubin metabolism (Goldman and Andrew, 2008). Bilirubin from heme is excreted in the urine or feces after it is conjugated to glucuronides in the liver.*

1.3. Disposition of Bilirubin by the Liver

Bilirubin excretion from the body is a major function of the liver, wherein specialized microanatomy enhances extraction of tightly protein-bound compounds from the circulation.
Hepatic translocation of bilirubin from blood to bile involves four distinct steps: (1) uptake of unconjugated bilirubin, by both an incompletely characterized facilitated transport process and by diffusion; (2) intracellular binding, mainly to various cytosolic proteins of the glutathione-*S*-transferase family; (3) conversion of unconjugated bilirubin to bilirubin monoglucuronides and diglucuronides by a specific uridine diphosphate (UDP)-glucuronosyltransferase isoform designated UGT1A1; and (4) transfer of bilirubin monoglucuronides and diglucuronides into bile by a canalicular membrane adenosine triphosphate (ATP)-dependent transporter designated multidrug resistance–associated protein 2 (MRP2) or canalicular multispecific organic anion transporter (cMOAT). MRP2/cMOAT is a member of the *MRP* gene family, other members of which pump certain drug conjugates, as well as unmodified anticancer drugs, out of cells (Goldman and Andrew, 2008).
Conjugation to bilirubin monoglucuronides and diglucuronides greatly increases the aqueous solubility of bilirubin and thereby enhances its elimination from the body while simultaneously reducing its ability to diffuse across biologic membranes, including the blood-brain barrier.

In newborns, a decreased capacity to conjugate bilirubin leads to unconjugated hyperbilirubinemia (physiologic jaundice of the newborn). If severe, this hyperbilirubinemia may lead to irreversible central nervous system toxicity. Phototherapy by exposure to blue light converts bilirubin to water-soluble photoisomers that are readily excreted in bile, protecting the central nervous system from bilirubin toxicity. Gilbert's syndrome and Crigler-Najjar syndrome types 1 and 2, which result from genetic defects in bilirubin conjugation, are characterized by unconjugated hyperbilirubinemia; in contrast, Dubin-Johnson syndrome, which results from inheritable defects in MRP2/cMOAT, is characterized by conjugated or mixed hyperbilirubinemia (Goldman and Andrew, 2008).

1.4. Metabolism of Lipids in normal and diseased Liver

Lipids are transported in blood as lipid-protein complexes called lipoproteins, which are classified based on their density and charges. The High-density Lipoprotein cholesterol (HDL-c) transports lipids out of peripheral tissues to the liver, while the Low Density Lipoproteins cholesterol (LDL-c) mobilizes lipids against the cells and blood vessels.

Triacylglycerols have been found to be elevated along with total cholesterol elevation. Therefore, elevated LDL-c, triacylglycerols and total cholesterol with reduced HDL-c will enhance the development of atherosclerosis and related cerebrovascular disorders (Aviram, 1993). LDL oxidation can lead to an additional atherogenic modification of lipoproteins, i.e., LDL aggregation. Aggregated LDL is taken up by macrophages at enhanced rate, leading to cellular cholesterol accumulation and foam cell formation which is the hallmark of atherosclerosis.

Cardiovascular diseases present some of the main health problems across the globe. The major ones are coronary heart diseases, stroke and hypertension (Aviram, 1993). Elevated plasma lipids are risk factors in cardiovascular problems. Hyperlipidaemia and other abnormal blood lipid profile are largely of genetic origin or due to unwholesome nutritional habits.

Lipids and other substances accumulate on arterial wall, forming plague, which occlude the vascular lumen and obstruct the blood flow to vital organs such as the heart, brain, liver, or kidney. Obstruction of blood supply to the heart, brain, liver or kidney causes coronary heart diseases, stroke or kidney failure, as the case may be. The important lipids whose elevations are implicated in these disease conditions are cholesterol and triacylglycerols.

Chronic parenchymal liver disease is associated with relatively predictable changes in plasma lipids and lipoproteins. Some of these changes are related to a reduction in the activity of Lethicin cholesterol acyltransferase (LCAT) (Mutter *et al*., 2010). This plasma enzyme is synthesized and glycosylated in the liver; then enters the blood, where it catalyzes the transfer of a fatty acid from lecithin to the free cholesterol to produce cholesterol ester and lysolecithin. As expected, in severe parenchymal liver disease, in which LCAT activity is decreased, plasma levels of cholesterol ester are reduced and free cholesterol level becomes normal or increased.

Plasma triacylglycerols are normally cleared by peripheral lipases (lipoprotein lipase or LPL and hepatic triglyceride lipase or HTGL). Because the activities of both LPL and HTGL are reduced in patients with hepatocellular disease, a relatively high level of plasma triacylglycerols may be found in both acute and chronic hepatitis, in patients with cirrhosis of the liver, and in patients with other diffuse hepatocellular disorders (Goldman and Andrew, 2008). With low LCAT activity and the elevated triacylglycerol level described, low-density lipoprotein (LDL) particles have an abnormal composition. They are relatively triacylglycerol rich and cholesterol ester poor.

High-density lipoprotein (HDL) metabolism may be abnormal in chronic liver disease as well. For example, because the conversion of HDL_3 (less antiatherosclerotic) to HDL_2 (more antiatherosclerotic) is catalyzed by LCAT, the reduced activity of LCAT in patients with cirrhosis leads to a decrease in the HDL_2:HDL_3 ratio (Goldman and Andrew, 2008). Conversely, the conversion of HDL_2 to HDL_3 requires hepatic lipases. If the activity of this lipase is reduced, one would expect an elevation in the HDL_2:HDL_3 ratio. Because the HDL_2:HDL_3 ratio is usually elevated in cirrhosis, the lipase deficiency appears to be the more dominant of the two mechanisms. These changes may result in an overall increase in serum total HDL levels (Mutter *et al*., 2010). How this affects the efficiency of the reverse cholesterol transport mechanism and the predisposition to atherosclerosis is not fully understood.

With regard to triacylglycerol levels in patients with severe parenchymal liver disease, the hepatic production of the triacylglycerol-rich, very-low-density lipoprotein (VLDL) particle is impaired. Yet the total level of plasma triacylglycerols remains relatively normal because the LDL particle in such patients is triacylglycerol- rich, for reasons that have not been fully elucidated (Goldman and Andrew, 2008).

Non-esterified fatty acid (NEFA) levels are elevated in patients with cirrhosis.
This change might be expected because basal hepatic glucose output is low in these patients. As a result, more NEFA are presumably required (via increased lipolysis) to meet the fasting energy requirements of peripheral tissues.

1.5. Oxidative stress and mechanism of hepatotoxicity

Liver plays a central role in transforming and clearing chemicals and is consequently susceptible to the toxicity induced from different agents. Chemicals that cause liver injury are termed hepatotoxins, and more than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which may manifest by abnormal liver enzyme tests (Jeong *et al*., 2002).

Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity.

The unique property of the liver to metabolize substances and its close relationship with the gastrointestinal tract makes it a highly susceptible organ to injury from drugs and other substances. Approximately 75% of blood reaching the liver arrives directly from gastrointestinal organs and then spleen through portal veins which bring drugs and xenobiotics in concentrated form. Numerous mechanisms may be cited to be responsible for either inducing hepatic injury or worsening the damage process (Chang and Schiano, 2007). Although the exact mechanism of hepatic injury remains largely unknown, it appears to involve two path-ways (1) direct hepatotoxicity and (2) adverse immune reactions.

In most instances, hepatic injury is initiated by the bioactivation of drugs to chemically reactive metabolites (Lee, 2003), which have the ability to interact with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage, and oxidative stress. Additionally, these reactive metabolites may induce disruption of ionic gradients and intracellular calcium stores, resulting in mitochondrial dysfunction and loss of energy production (Lynch and Price, 2007). Its dysfunction releases excessive amount of oxidants which in turn injures hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress (Jeong *et al*., 2002).

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen that build up oxidative stress. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are often generated as byproducts of biological reactions or from exogenous factors. In vivo, some of these ROS play positive roles in cell physiology; however, they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations leading to cancer, degenerative, and other diseases (Cerutti, 1991; Finkel and Holbrook, 1998).

Evidences developed over the last years have suggested that various forms of liver injuries may also be caused by free radical formation and subsequent oxidative stress. It is believed that reactive oxygen species (ROS), such as hydroxyl radical, superoxide radical anion and nitric oxide may injure cell membranes through lipid peroxidation (Valko *et al*., 2007).
This oxidative stress lead to the formation of glycoxidation products, including advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs). The receptor for advanced glycation end products (RAGE) is a signal transduction receptor that binds both AGEs and AOPPs. RAGE is expressed by hepatic stellate cells and myofibroblasts, which are the relevant cells for fibrogenesis of chronic liver disease. Both AGEs and AOPPs trigger the inflammatory response *via* interaction with RAGE and by causing activation of nuclear factor kappa B (NF-κB) (Hyogo and Yamagishi, 2008).
Advanced oxidation protein products are not only markers of oxidative stress, but also act as inflammatory mediators (Fialova *et al*., 2006).

The knowledge of AOPPs pathophysiology in chronic liver disease could provide valuable information with respect to the relationship between oxidative stress and the inflammatory response related to liver fibrosis (Zuwała *et al*., 2011). Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver which concomitantly promotes further liver damage. This impairment of cellular function can culminate in cell death and possible liver failure. Hepatic cellular dysfunction and death also have the ability to initiate immunological reactions, including both innate and adaptive immune responses (Blazka *et al.,* 1995).
Stress and damage to hepatocytes result in the release of signals that stimulate activation of other cells, particularly those of the innate immune system, including Kupffer cells (KC) and natural killer T cells (NKT). These cells contribute to the progression of liver injury by producing proinflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver (**figure 1.2**).

It has been demonstrated that various inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β, produced during hepatic injury are involved in promoting tissue damage however, innate immune cells are also the main source of IL-10, IL-6, and certain prostaglandins, all of which have been shown to play a hepatoprotective role (Bourdi *et al.,* 2002). Thus, it is the delicate balance of inflammatory and hepatoprotective mediators produced after activation of the innate immune system that determines an individual's susceptibility and adaptation to hepatic injury (Rubinstein *et al.,* 1986).

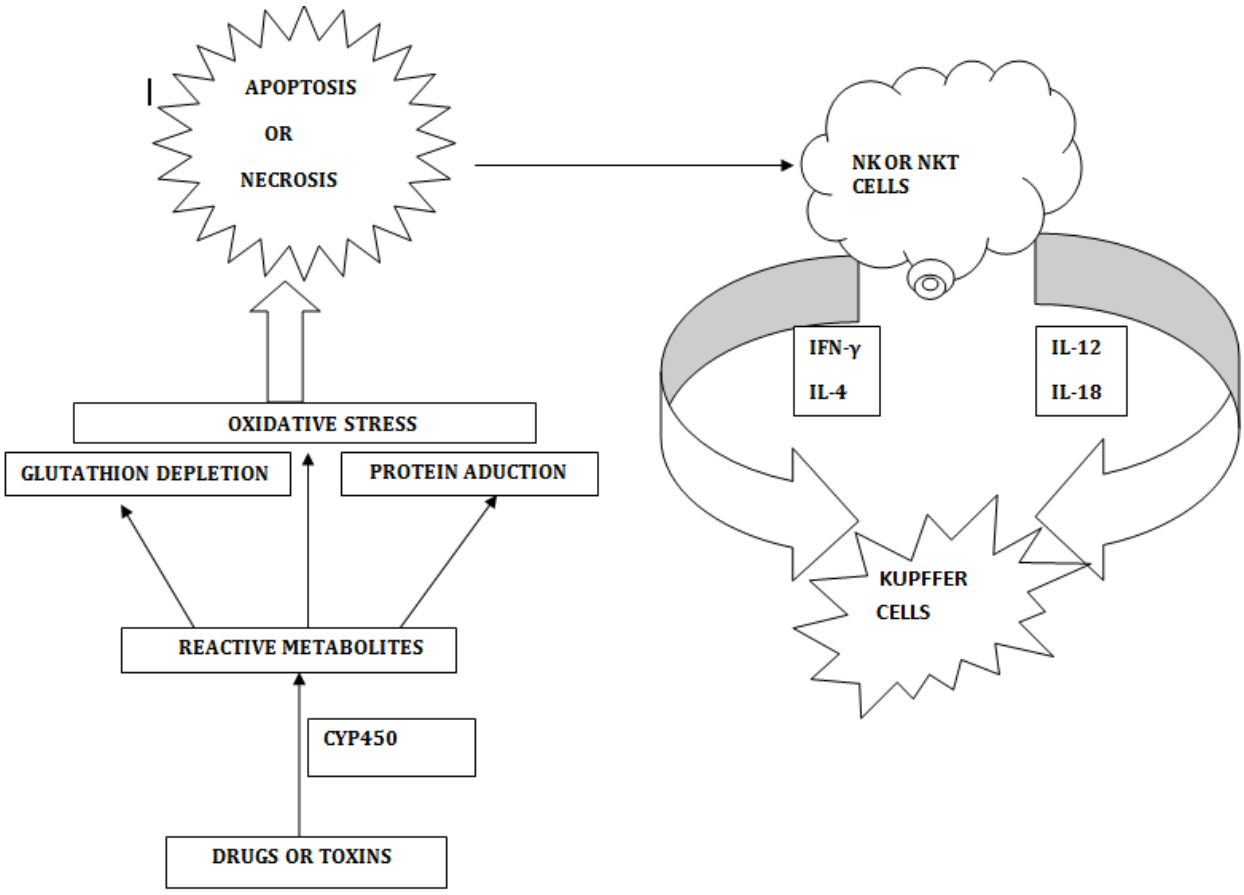


Figure 1.2 *Mechanism of hepatic injury after toxins or drugs are converted in to reactive metabolites by cyp450 enzyme.*

Liver injury may follow the inhalation, ingestion, or parenteral administration of a number of pharmacologic and chemical agents. These include industrial toxins (e.g., carbon tetrachloride, trichloroethylene, and yellow phosphorus), Allopurinol, lovastatin, Amiodarone, Phenytoin, the heat-stable toxic bicyclic octapeptides of certain species of *Amanita* and *Galerina* (hepatotoxic mushroom poisoning), and, more commonly, pharmacologic agents used in medical therapy (Chang and Schiano, 2007).

It is essential that any patient presenting with jaundice or altered biochemical liver tests be questioned carefully about exposure to chemicals used in work or at home, drugs taken by prescription or bought "over the counter," and herbal or alternative medicines.

Hepatotoxic drugs can injure the hepatocyte directly, e.g., via a free-radical or metabolic intermediate that causes peroxidation of membrane lipids and that results in liver cell injury (Chalasani *et al*., 2008). Alternatively, the drug or its metabolite can distort cell membranes or other cellular molecules, bind covalently to intracellular proteins, activate apoptotic pathways, interfere with bile salt export proteins, or block biochemical pathways or cellular integrity.

Interference with bile canalicular pumps can allow endogenous bile acids, which can injure the liver, to accumulate. Such injuries, in turn, may lead to necrosis of hepatocytes; injure bile ducts, producing cholestasis; or block pathways of lipid movement, inhibit protein synthesis, or impair mitochondrial oxidation of fatty acids, resulting in lactic acidosis and intracellular triglyceride accumulation (expressed histologically as microvesicular steatosis).

In some cases, drug metabolites sensitize hepatocytes to toxic cytokines, and differences between susceptible and non susceptible drug recipients may be attributable to polymorphisms in elaboration of competing, protective cytokines, as has been suggested for acetaminophen hepatotoxicity (Lee, 2003). Immunologically mediated liver injury has been postulated to represent another mechanism of drug hepatotoxicity. In addition, a role has been shown for activation of nuclear transporters, such as the constitutive androstane receptor (CAR), in the induction of drug hepatotoxicity.

Most drugs, which are water-insoluble, undergo a series of hepatic metabolic transformation steps, culminating in a water-soluble form appropriate for renal or biliary excretion (Chalasani *et al*., 2008) and this process begins with oxidation or methylation initially mediated by the microsomal mixed-function oxygenases cytochrome P450 (phase I reaction), followed by glucuronidation or sulfation (phase II reaction) or inactivation by glutathione. Most drug hepatotoxicity is mediated by a phase I toxic metabolite, but glutathione depletion, precluding inactivation of harmful compounds by glutathione S-transferase, can contribute as well (Lynch and Price, 2007).

Direct toxic hepatitis occurs with predictable regularity in individuals exposed to the offending agent and is dose-dependent. The latent period between exposure and liver injury is usually short (often several hours) although clinical manifestations may be delayed for 24–48 h.

Agents producing toxic hepatitis are generally systemic poisons(Lee, 2003) or are converted in the liver to toxic metabolites.

The direct hepatotoxin results in morphologic abnormalities which are reasonably characteristic and reproducible for each toxin. For example, carbon tetrachloride and trichloroethylene characteristically produce a centrilobular zonal necrosis, whereas yellow phosphorus poisoning typically results in periportal injury.

The hepatotoxic octapeptides of *Amanita phalloides* usually produce massive hepatic necrosis; the lethal dose of the toxin is ~10 mg, the amount found in a single death cap mushroom (Schmidt *et al*., 2002). Tetracycline, when administered in IV doses >1.5 g daily, leads to microvesicular fat deposits in the liver. Liver injury, which is often only one facet of the toxicity produced by the direct hepatotoxins, may under go unrecognized until jaundice appears.

1.6. Carbon tetrachloride induced-hepatotoxicity

Reactive oxygen species (ROS) generated by some toxicants can cause oxidative stress, lipid peroxidation, and oxidative tissue damage.

Carbon tetrachloride (CCl_4), a well-known generator of reactive species and a hepatotoxicant that causes acute liver injury, has been used to study liver pathology associated with oxidative stress and free radicals. CCl_4-induced hepatic steatosis is a well-known experimental model used to screen hepatoprotective agents (Recknagel and Glende, 1973).

Toxicity of CCl_4 has been extensively studied. During the metabolism of CCl_4 by the mitochondrial monooxygenase (P450 2E1) system in the endoplasmic reticulum of liver, an unstable metabolite, trichloromethyl free radical ($\cdot CCl_3$-) is generated, then ($\cdot CCl_3O$-) radical is formed and these are rapidly converted to trichloromethyl peroxide (Cl_3COO-),(Parola *et al.*,1992; Recknagel *et al.,*1989). These radicals ($\cdot CCl_3$-), ($\cdot CCl_3O$-), and (Cl_3COO-) initiate lipid peroxidation mainly in microsomes (i.e., endoplasmic reticulum) and poly unsaturated fatty acids (PUFA) of liver cell membranes, and lead to liver injury processes such as steatosis.

Hepatocyte membrane damage causes alterations in lipoprotein secretion and accumulation of lipoprotein and lipid droplets in hepatocytes (Cotran *et al*., 1999; Wang *et al*., 2008). Despite the elimination of CCl_4 within 24 h, because of its volatility and liver injury progresses, elimination of free radicals and prevention of lipid peroxidation is important in the prevention and treatment of hepatic damage (Junnila *et al*., 2000).

The effects of CCl_4 on hepatocytes, depending on dose and exposure time, are manifested histologically as hepatic steatotis (e.g. fatty infiltration), centrilobular necrosis and ultimately cirrhosis (Kumaravelu *et al.,* 1995). The damage or death of tissue usually results in the leakage of the enzymes in the affected tissue(s) into the blood stream.

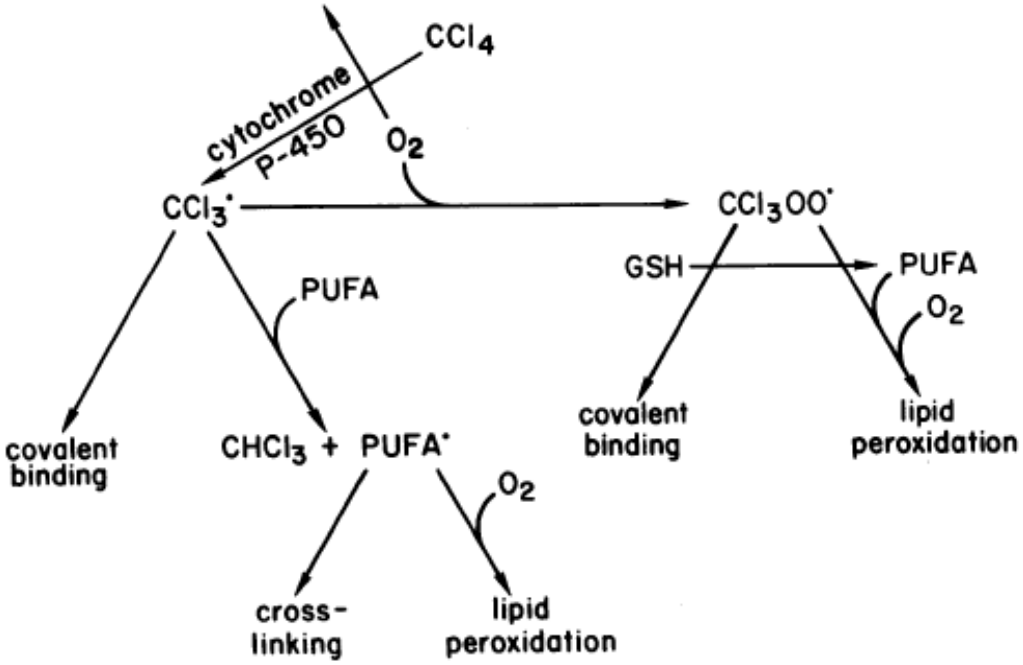


Figure1.3 *Carbon tetrachloride and membrane damage after it is converted in to free radicals in liver microsomes (Burk, 1984).*

The polyhalogenated compound $CC1_4$ is a well-known hepatotoxin and exposure to this chemical is known to result in hepatocellular necrosis in rodents (Rao and Mehendale, 1989). Carbon tetrachloride (CCl_4), as a Xenobiotic, causes oxidative stress and may injures hepatic cells.

It has been widely accepted that liver injury caused by CCl_4 depends on its metabolism to highly reactive trichloromethyl free radical (·CCl_3-) which interacts with molecular oxygen to form the trichloromethyl peroxy radical (Mac Cay *et al.*, 1984 and Poli *et al*.,1985). Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so playing a significant role in the pathogenesis of diseases (Recknagel *et al*., 1989).
The biotransformation of CCl_4 to metabolites is a cytochrome P450 mediated reaction that initiates lipid peroxidation and attendant tissue damage.

The metabolic effects of CCl_4 inside mitochondria have been described (Brattin *et al*., 1984) and it has been found that damage to the calcium pump in mitochondria (Albano *et al*., 1985) is dependent upon haloalkylation. However, the profound accumulation of fat following CCl_4 poisoning is considered to be independent of mitochondrial damage.

The fatty infiltration of the liver is thought to develop as a result of the action of free alkyl radicals on biomembranes that in turn cause halo alkylation-dependent blocking at the exit of the lipoprotein micelles from the Golgi apparatus (Poli *et al*., 1987). For this reason, anti-oxidation is an extremely significant activity which can be used as a preventive agent against diseases. In spite of tremendous advances in modern medicine, there are no effective drugs available to improve liver function, offer protection or help to regenerate hepatic cells.

1.7. Medicinal plants of the World

From the earliest times, herbs have been prized for their pain-relieving and healing abilities and today we still rely largely on the curative properties of plants.
According to World Health Organization, 80 % of the people living in rural areas depend on medicinal herbs as primary healthcare system. The synthetic anticancer remedies are beyond the reach of common man because of cost factor (Deshmukh *et al*., 2011). Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical.

A great deal of pharmaceutical research done in technologically advanced countries like USA, Germany, France, Japan and China has considerably improved quality of the herbal medicines used in the treatment of cancer. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Certain biological response modifiers derived from herbs are known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes. Some herbs reduce toxic side effects of chemotherapy and radiotherapy.

Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. By understanding the complex synergistic interaction of various constituents of anticancer herbs, the herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body (Deshmukh *et al*., 2011).

Medicinal herbs are also significant source of synthetic and herbal drugs. So far, pharmaceutical companies have screened more than 25,000 plants for anti-cancer drugs. This should tell us that looking for single ingredients to attack cancer might be missing the point. Just as diseases are a product of disturbances in the body, so herbs can correct the disturbances as well as control many diseases.

Herbal system of medicine has been practiced for thousands of years. Phytoconstituents derived from the herbs have been used in various formulations to enhance activity of immune cells of the body that promotes production of cytokines including interleukin, interferon, tumor necrosis factor and colony stimulating factor. These formulations help the body to fight cancer more effectively and reduce toxic side effects of chemotherapy and radiotherapy stages of cancer.

Medicinal plants have been acknowledged and are extremely valued all over the world as a prosperous source of bioactive for the prevention and treatment of ailments. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and minimal side effects. Ancient literatures also mention herbal therapy for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is yet available (Deshmukh *et al*., 2011).

The herbal drug products are prepared from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses growing these raw materials (Brower, 1998). Recently, World Health Organization (WHO) defined traditional medicine (including herbal drugs) as therapeutic practices that have been in existence, often for hundreds of years before the development and spread of modern medicine and are still in use today (Jeong *et al*., 2002). These practices incorporated ancient beliefs and were passed on from one generation to the next by oral tradition and/or guarded literatures. Therefore, these plant drugs deserve detailed studies in the light of modern science.

Naturopaths and others, who look beneath the symptoms of an illness to its underlying cause, often discover that the liver has a role to play. More than 500 vital functions have been identified with the liver and a person's nutritional level is not only determined by what he eats, but also by what the liver processes.

Unfortunately, it is extremely difficult to detect early warning symptoms specific to liver metabolic imbalances and a person may suffer for a long time from a liver disorder without knowing it.

The incredible complexity of liver chemistry and its fundamental role in human physiology is so daunting to researchers that they visualize that perhaps simple plant remedies might have something to offer is astonishing and incredible. In fact, nature has bestowed some plants with the property to prevent, treat and cure hepatic disturbances with interception of fewer side effects.

Hepatoprotectives are a class of therapeutic agents that include synthetic as well as natural product which offer protection to liver from damage or help in regeneration of hepatic cells. Medicinal herbs are significant source of hepatoprotective drugs. It has been reported that about 170 phytoconstituents isolated from 110 plants belonging to 55 families do possess hepatoprotective activity.

Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, curcuminoids, lignans, essential oils and terpenoids (Handa, 1991). Clinical research has also shown that herbals have genuine utility in the treatment of liver diseases. Only a small portion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficacy (Trease and Evans, 1983).

1.7.1. Medicinal plants and their uses in Ethiopia

Plants are the principal sources of raw materials for plant-based medicine since ancient times. Of late, the traditional herbal medicines are receiving great importance in the health care sector. Each and every tribal/ethnic community has its own system of traditional medicine and they utilize natural resources around their habitats for various medicinal purposes. The traditional knowledge is handed down orally from one generation to the other through trial and error method (Fassil, 1998).

Large sections of the rural population living far away from urban area still rely on traditional herbal medicines for their health care needs. This is because of the lack of primary health care center and transportation facilities. Besides, medicinal plants are easily available natural products, easily formulatable and cost-effective with negligible or no side-effects.

The cultivation and use of spices, herbs, medicinal and other essential oil bearing plants is not new to Ethiopia. It is as old as the crop themselves, and its history can be traced back to the reign of Queen Sheba (*ca*.992 BC). Ethiopia is the origin and/or center of diversity for many of these plant species (Lambert, 2006).

Various literatures show the significant role of medicinal plant in primary health care delivery in Ethiopia where 70% of human and 90% of livestock population depend on traditional medicine similar to many developing countries particularly that of Sub-Saharan African countries. The traditional health care is culturally deep rooted with oral and written pharmacopoeias. Ethiopian plants have shown very effective medicinal value for some ailments of human and domestic animals thus medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs throughout the country (Ensermu *et al*., 1992).

The major reasons why medicinal plants are demanded in Ethiopia are due to culturally linked traditions, the trust the communities have in the medicinal values of traditional medicine and relatively low cost in using them (Hareya, 2005). This is acknowledged for example by a result of recent study that was conducted and showed that the value of both imported and domestically produced pharmaceutical products were about ETB 1.05 billion. During the same year the value of medicinal plants including traded and non traded ones was ETB 423 million making average health coverage of 42% of Ethiopian expenditure on pharmaceutical products. This is not only a significant saving in terms of foreign currency but also availability assurance for the primary health care systems in the rural community (Hareya, 2005).

The volume of sales of medicinal plants has increased over years and this has been taken by some as the existence of disease prevalence requiring traditional Medicare causing increased demand which led to harvesting important medicinal plants. The expected increase in the cost of commercial drugs and their occasional impotency also increase demand of medicinal plants. The proportion of consumers who rely on harvesting medicinal plant is the highest in the rural area, since collecting from natural plantation is most accessible and cost- effective.

There are 6500 species of higher plants in Ethiopia making the country one of the most diverse floristic regions in the world. There are large numbers of moderate to high value medicinal plants, herbs and spices existing in the wild. However, of the existing medicinal herbs and spice plants only small percent are traded (Dawit and Estifanos, 1991).

Being a land of diverse climatic and edaphic potentials, several of such indigenous and exotic species and essential oil bearing plants could luxuriously grow in Ethiopia and provide remarkable benefits to the national economy (Lambert, 2006). However, so far only two of the major 18 agro-ecological zones defined as hot to warm humid lowlands and tepid to cool humid midlands carry out some research and production activities of some herbs, spices and related aromatic plants.

The research made so far on Ethiopian medicinal plants has been mostly of producing inventories and checklists, some have been touched by modern research where their principal component has been analyzed and defined (Fassil, 1998).

The bulk of the plant matter used for medicinal purposes is collected from natural vegetation stocks that are shrinking with degraded environment and is faced to substantial reduction or dwindling of species of medicinal plants. Ethiopia is a rich source of medicinal plants. Perhaps, the best known species is *Phytolacca dodecandra*. Extracts of the plant, commonly known as endod, are used as an effective molluscicide to control schistosomiasis (Aklilu, 1991).

The medicinal plants of Ethiopia and the developing countries play major supplementary roles to the limited modern health care available. The development of useful and widely used drugs like Digoxin and Digitoxin, from Digitalis leaves; quinine from the cinchona bark; reserpine from *Rauwolfia serpentine*; morphine from *Papaver somniferum*; cocaine from *Erythroxzion coca* and the anti cancer *Vincristiner* and *Viblastine* from *Cartharathus troseus* of Madagascar and again anti-cancer compound, bruceatin, from the Ethiopian plant, *Brucea antidysentrica*, just to name a few, are examples of the contributions of traditional pharmacopoeia (Desta, 1984).

Those plants are part of the economic commodity for some members of the society which make their livelihood on their collection, trade and medicinal practices by practitioners or healers. It thus has a substantial potential to make contributions to the economic growth and alleviation of poverty in the country. Its proper management protect environment and conserve biodiversity.

The current demands for herbal remedies in both developed and developing countries is increasing. In developed countries this may be partly due to the dissatisfaction with conventional medicines while with the developing countries this is due to lack of medical doctors, shortage of pharmaceutical products and their unaffordable prices.

In addition, the use of modern drugs to treat AIDS, cancers, and chronic complaints such as rheumatism, arthritis and asthma have been realized. Whatever the reasons, it cannot be denied that herbal remedies are currently enjoying widespread popularity throughout the world.

According to Ensermu *et al*., 1992 and Edwards, 2001, habitat and species are being lost rapidly as a result of the combined effects of environmental degradation, agricultural expansion, deforestation and over harvesting of species and this is further enhanced by human and livestock population increase thus hastening the overall rural livelihood impoverishment and loss of the biological diversity and indigenous knowledge which is also of global concern since some of this are endemic to Ethiopia (Abdulhamid and Sebsib, 2004), and a full scale plan to conserve, develop and effectively utilize this resource needs investment commitments by government agencies, the private sector, and various global foreign aids for development. However, before such investments and support are realized, a clear indication of the resource condition and its economic values must be worked out. This needs a critical overview of medicinal plants in Ethiopia, their demands, trade, and economic benefits. Such an overview has to come up with a formulation of the strength, weakness and opportunities in the medicinal plant sector to forward conclusions and recommendation.

1.7.2. Antioxidant activities of some medicinal plants

In absence of reliable liver-protective drugs and in view of severe undesirable side effects of synthetic agents, there is growing need to utilize abundant plant resources available and to evaluate scientific basis for the medicinal plants that are claimed to possess hepatoprotective activity and therefore, attention is given to natural antioxidants. Natural antioxidants are polyphenolic compounds which are found in all plants (Kikuzaki *et al*., 1991). Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (Devasagayam *et al*., 2004).This has attracted a great deal of research interest in natural herbs. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased (Deiana *et al*., 1999; Wang and Jiao, 2000). Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, turmeric, white pepper, chili pepper, ginger, and other several medicinal plants extracts (Kikuzaki *et al*., 1991).

The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins.
In addition to the above compounds found in natural foods, vitamins C and E, b-carotene, and a-tocopherol are known to possess antioxidant potential (Prior, 2003). A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported (Kaur and Kapoor, 2002).Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can reduce the risk of heart disease (Landbo A and Meyer, 2001).

Since ancient times, the medicinal properties of plants have been investigated due to their potent antioxidant activities. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers (Kaur and Kapoor, 2002).

The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Prior, 2003).

This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process. Besides, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc.
Crude extracts of herbs, spices and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. While, flavonoids are a group of polyphenolic compounds with known properties including free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Kikuzaki *et al*., 1991).

Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury.

Although several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commercially available, but are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants.

Natural antioxidants especially phenolics and flavonoids from tea, wine, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or as nutritional supplements. Also many other plant species have been investigated in the search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

1.7.3. Ginger and its Medicinal uses

The rhizome of ginger has been widely distributed in the tropic and semi-tropic regions of the world. Ginger has an ancient history. It is possible that as long as 5,000 years ago, spice caravans were carrying dried ginger from India to the Middle East (Mascolo *et al., 1989*). Ginger is one of the best known of spices and it is believed that its' medicinal properties have been known in China for thousands of years. Indeed, the plant was mentioned in Emperor Shen Hung's Pen Tsao Ching (The Classic Book of Herbs), which he wrote in 3000 BCE. Although frequently associated with Traditional Chinese medicine, ginger has an ancient reputation in India and is specifically mentioned in the 'Ayurveda', the Hindu manual of medicine written in the fifth century BCE. An ancient Indian proverb states: Every good quality is contained in Ginger.

The ancient Greeks - such as Galen knew and used ginger. Inheriting the Greek traditions and knowledge, the ancient Romans also valued ginger for its' culinary and medicinal uses. For example, Largus, a physician in the Roman army and the author of a book entitled De Compositione Medicamentorum, described the Roman military expedition to Britain and was responsible for the introduction of Opium and ginger to the island. Another Roman, Pedianos Dioscorides, wrote about Ginger in his famous De Materia Medica (77 CE). He was concerned to ensure a 'better quality of drug and mentioned for example, some of the problems associated with the storage of Ginger (Mascolo *et al., 1989*).

Subsequently, Ginger received a mention in the Islamic holy book, the Koran where it says that among the righteous in Paradise are passed vessels of silver and goblets of glass and a cup, the admixture of which is Ginger. Later, when the spice came to medieval Europe, it was thought that it had come from the Garden of Eden.

In the middle ages in England, just one pound of the spice was held to be equal in value to a sheep and for a long time only the wealthy could afford to use it. Queen Elizabeth I used it as a digestive aid. During the 15th century, Spanish explorer called Francisco de Mendosa transplanted the Ginger plant and brought it back to Spain from the East Indies. Thereafter, following the arrival of Spanish explorers and settlers to the North American continent, from that on words ginger was soon introduced and became naturalized there. Chinese ships carried pots of Ginger on board long sea voyages to prevent scurvy and sea sickness. A Chinese folk remedy recommends rubbing the cut root of the plant on the scalp to stop hair loss.

In India, before religious festivals, devotees would avoid Garlic, so as not to offend the deities. Instead, they consumed Ginger, which would leave them fragrant and pleasing. A product of India, Ginger is considered the universal Medicine by India's Ayurvedic herbalists (Mascolo *et al., 1989*). Ginger Root has also been used for centuries in Chinese herbal medicine for the positive effects it has on the body, as well as to enhance herbal combinations. Ginger's sweet taste has made it a popular herb, and it is found today in ginger ale, breads, candies, and tonics.

1.7.3.1. History of ginger in Ethiopia

The cultivation of ginger started in Ethiopia during 13th century when Arabs introduced it from India to east Africa (Hailemichael and Tesfaye, 2008). In Ethiopia it is limited mostly in the wetter regions of Southern Nations, Nationalities and Peoples Regional State (SNNPRS) and some parts of western Oromia. Most of the commercial production is practiced in SNNPRS by farmers within Kambata-Tambaro, Wolaita and Hadiya zones.

Ginger is commercially produced as horticultural crop in SNNPS, Ethiopia. The producing areas in SNNPRS are said to be **"Ginger Belts in Ethiopia"** where much of the country's production and marketing activities are located. Even though these areas were known as major suppliers of ginger in the country, limited attention has been given to expand production for betterment of smallholder farmers engaged in production and marketing activities.

Efforts to generate improved technologies were limited to agronomic practices with no concerted effort to improve product quality, which is highly influenced through its value chain from pre-harvest to postharvest management practices including processing of final products. Moreover, no effort has been made to assess farmers' pre and post-harvest management practices that could be used as a benchmark for improvement works targeting product quality and sustainable supply.

Factors that determine performance of ginger marketing and reduce benefits to farmers engaged in its production were not adequately understood by policy makers and agricultural extension workers (Hailemichael and Tesfaye, 2008).

1.7.3.2. Ethno botanical description of Ginger

Ginger, a very useful herb plant, is believed to originated in India, China and Java, yet is also native to Africa. It is grown throughout the tropical areas of the world.

Ginger is scientifically named as *Zingiber officinale Roscoe*, after an English botanist, William Roscoe named the plant as *Zingiber officinale* in his publication in 1807.

The stem is surrounded by the leaves. It shoots up a stem with narrow spear-shaped leaves, as well as white or yellow flowers growing directly from the root.

Figure1.3. *Ginger plant with its rhizome*

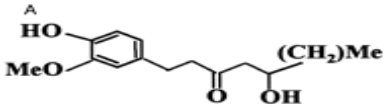
The word Ginger comes from the ancient Sanskrit word"*Singabera*" meaning shaped like a horn. Ginger oleoresin and ginger oil is derived from the fleshy part of the mesocarp of the herbs species. Ginger is a tropical herbaceous perennial with underground rhizomes from which stalks arise three feet tall. The inflorescence comes directly from the roots (rhizomes) and ends in a spilled. The flower has an aromatic smell. Nowadays ginger is commercially cultivated in nearly every tropical and subtropical country in the world with arable land to produce the valuable polyphenolic compounds or oleoresins.

1.7.3.3. Phytochemical Constituents of Ginger

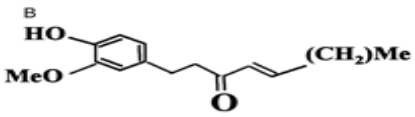
The chemical constituents and biological (pharmacological) activities of the rhizome of Zingiber species have been studied intensively. The volatile oil chemical constituents make up about 1.0 – 3.3% by mass weight of the dry Zingiber species rhizome. More than 140 compounds have been separated and about 75 of them have been identified using gas chromatography – mass spectrometry (GC-MS). 30 – 70% of the volatile oil fraction are sesquiterpene mainly, (-)-zingiberene, and zingiberol.

The aroma of ginger is largely the results of zingiberene and zingiberol. The ginger root also contains oleoresin (4.0 – 7.5% by mass weight of the rhizome) comprising non-volatile, pungent lipid soluble chemical constituents (Matsuda *et al.,* 2009). The main pungent compounds in fresh ginger are a series of homologous phenolic ketones known as 6-gingerol, while 8-gingerol and 10-gingerol occur in smaller quantities. Nevertheless, during storage or thermal processing, the gingerols may be modified to a series of homologous compounds called 6-, 8-, 10-shagaol (Matsuda *et al.,* 2009). Shagaols, which are more pungent than gingerol, are the major pungent compounds in dried ginger rhizome.

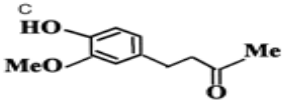
The plant also contains starch (50%), protein (9%), lipids (including glycerides, phosphatidic acid, lecithins, and fatty acids; 6-8%) and protease (2.26%), mucilage, Proteolytic enzyme (zingibain), Magnesium, Potassium, Linoleic cid, gum, starch, fiber(lignin), Calcium, Iron, Magnesium, Phosphorus, Protein, Sodium, Vitamins A, Vitamin B-complex and Vitamin C.



Gingerols



Shogaols



Zingerone

Figure 1.4 *Chemical structures of gingerol, shogaols and zingerone.*

1.7.3.4. Pharmacological properties of ginger

The use of natural products as an alternative to conventional treatment in the treatment of various diseases has been on the rise in the last few decades (Park and Pezzuto, 2002). In addition, the uses of natural antioxidant as a therapeutic adjuvant to improve organ damage induced by various agents have been developed.

According to glossary produced by American Diabetics Association, neutriceutics are substances considered as food or a part of it that offers health or medicinal benefit, including prevention and treatment of diseases (Park and Pezzuto, 2002). Some of the natural products find their use not as pharmaceuticals (real medicine) but as a novel class of dietary supplements or nutraceuticals that fall well into the concept of functional foods. Despite the favorable ethno pharmacological properties of ginger, its protective effect against liver fibrosis has not previously been explored and its role as diminished factor of fibrosis could be a marker of therapeutic benefit.

Ginger has been cultivated for thousands of years as a spice and for medicinal purposes (Park and Pezzuto, 2002).Chemical constituents like gingerols and shogaols present in ginger exhibited strong antihepatotoxic activity because it scavenges superoxide anion and hydroxyl radicals (Kikusaki and Nakatani, 1993). Gingerol, the pungent factor in ginger, inhibits phospholipid peroxidation induced by the $FeCl_3$ ascorbate system. Inhibition of xanthine oxidase activity responsible for the generation of reactive oxygen species, such as superoxide anion has been documented with gingerols.

The moderate pungency of ginger has been attributed to the mixture of gingerol derivatives in the oleoresin fraction of processed ginger (Mustafa *et al*., 1993). The major pungent compounds in ginger from studies of the lipophilic rhizome extracts have yielded potentially active gingerols which can be converted to shogaols, zingerone, and paradol up on drying (Govindarajan, 1982). It has been demonstrated that this phenolic substance has been found to possess many interesting physiological and pharmacological activities and is widely used in aromatherapy, medicine and as well as flavoring food and drink industries.

Along the antioxidant properties 6-gingerol also possesses useful (Aeschbach *et al*., 1994), anti-inflammatory (Mascolo *et al*., 1989) and anti-tumor promoting activities (Park *et al*., 2002). In addition, it has been reported that ginger has antipyretic, analgesic, and hypotensive effects (Surh *et al*., 1998).

1.7.3.5. Antioxidant effect of ginger

The balance between oxidation and antioxidation is believed to be critical in maintaining healthy biological systems (Kikusaki and Nakatani, 1993). Under physiological conditions, the human antioxidative defense system including e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and others, allows the elimination of excess reactive oxygen species (ROS) including, superoxide anions ($O_{2}^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), alkoxyl radicals ($RO^{\cdot}$) and peroxyradicals ($ROO^{\cdot}$) etc.

However, endogenous antioxidant defense systems are incomplete without exogenous reducing compounds such as vitamin C, vitamin E, carotenoids and polyphenols, playing an essential role in many antioxidant mechanisms in living organisms. Therefore, there is continuous demand for exogenous antioxidants in order to prevent oxidative stress, representing a disequilibrium redox state in favor of oxidation. However, high doses of isolated compounds may be toxic, owing to prooxidative effects at high concentrations or their potential to react with beneficial concentrations of ROS normally present at physiological conditions that are required for optimal cellular functioning.

Antioxidant is a substance that can fight and destroy excess free radicals and repair oxidative damage. Nowadays, the synthetic antioxidants are widely used in the food industry and also included in the human diet. The use of natural antioxidant is safer than synthetic antioxidants (Ali *et al*., 2008). Phytochemical compounds that benefit to health can be found in herbs, fruit and plants. Polyphenols are the main bioactive phytochemicals that have been shown to prevent many types of chronic diseases such as cancer and cardiovascular diseases.

The antioxidant activity of Zingiber officinale was shown to be as effective as vitamin C in lowering lipid per oxidation by influencing the enzymatic blood level of superoxide dismutase, catalase, and glutathione peroxidase. Ginger can significantly eliminate the superoxide anions generated by the hypoxanthine-xanthine oxidase system and the hydroxide free radicals produced by ultra-violate radiation on H_2O_2 or H_2O. Concentrated solution of ginger extract significantly enhances the activity of the superoxide dismutase of the liver.

The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of radiation (Ali *et al*., 2008), and a number of toxic agents such as carbon tetrachloride and cisplatin. Recently, it has been shown that [6]-gingerol is endowed with strong anti-oxidant action both in vivo and in vitro, in addition to strong anti-inflammatory and anti-apoptotic actions (Ali *et al*., 2008). This makes it a very effective agent for prevention of ultra violet B (UVB)-induced reactive oxygen species production and COX-2 expression, and a possible therapeutic agent against UVB-induced skin disorders.

The main antioxidant active principles in ginger are the gingerols and shogaols and some related phenolic ketone derivatives. It also possesses antioxidative characteristics, since it can scavenge superoxide anion and hydroxyl radicals. Gingerol from ginger inhibited, at high concentrations, ascorbate/ferrous complex induced lipid peroxidation in rat liver microsomes. Gingerol isolated from ginger was shown to inhibit platelet function due to inhibition of thromboxanes formation. Furthermore, ginger acts as a hypolipidemic agent inhibiting cholesterol biosynthesis (Bhandari *et al*., 1998; Sharma *et al*., 1996).

There is a causal relationship between inflammation and cancer resulted from oxidative stress, and iNOS and COX-2 are considered as potential molecular targets for chemoprevention (Pan, 2008). The possible mechanism through which 6-shogaol down regulates inflammatory iNOS and COX-2 gene expression in macrophages is inhibiting the activation of nuclear factor kappa (NF-κB) by interfering with the activation of PI3K and MAPK. Exposure of cells to oxidative and proinflammatory stimuli like lipopolysaccharides (LPS) causes activation of a series of upstream kinases such as MAPKs, IKK, PKC, and PI3K, which then activate NF-κB by phosphorylation-mediated degradation of IκBα. Activated upstream kinases also phosphorylate p65, the active subunit of NF-κB (Pan, 2008).

Free activated NF-κB, in the form of p65-p50 heterodimer, translocates to the nucleus. In resting cells, the NF-κB heterodimer is held in the cytosol through interaction with inhibitory IκB proteins (Pan, 2008), but exposure to proinflammatory stimuli cause IκBα to becomes phosphorylated, ubiquitinated, and then degraded. Thus, the liberated NF-κB dimmers are translocated to the nucleus, where the transcription of target gene is induced. Activated nuclear factor-κB (NF-κB) and AP-1 often facilitate transcription of numerous genes, including iNOS and COX-2, resulting in ROS and proinflammatory mediators.

6-shogaol is then found to inhibit nuclear translocation of NF-κB by suppressing phosphorylation of IκBα and p65 and subsequent nuclear translocation of subunits of NF-κB (Pan, 2008).

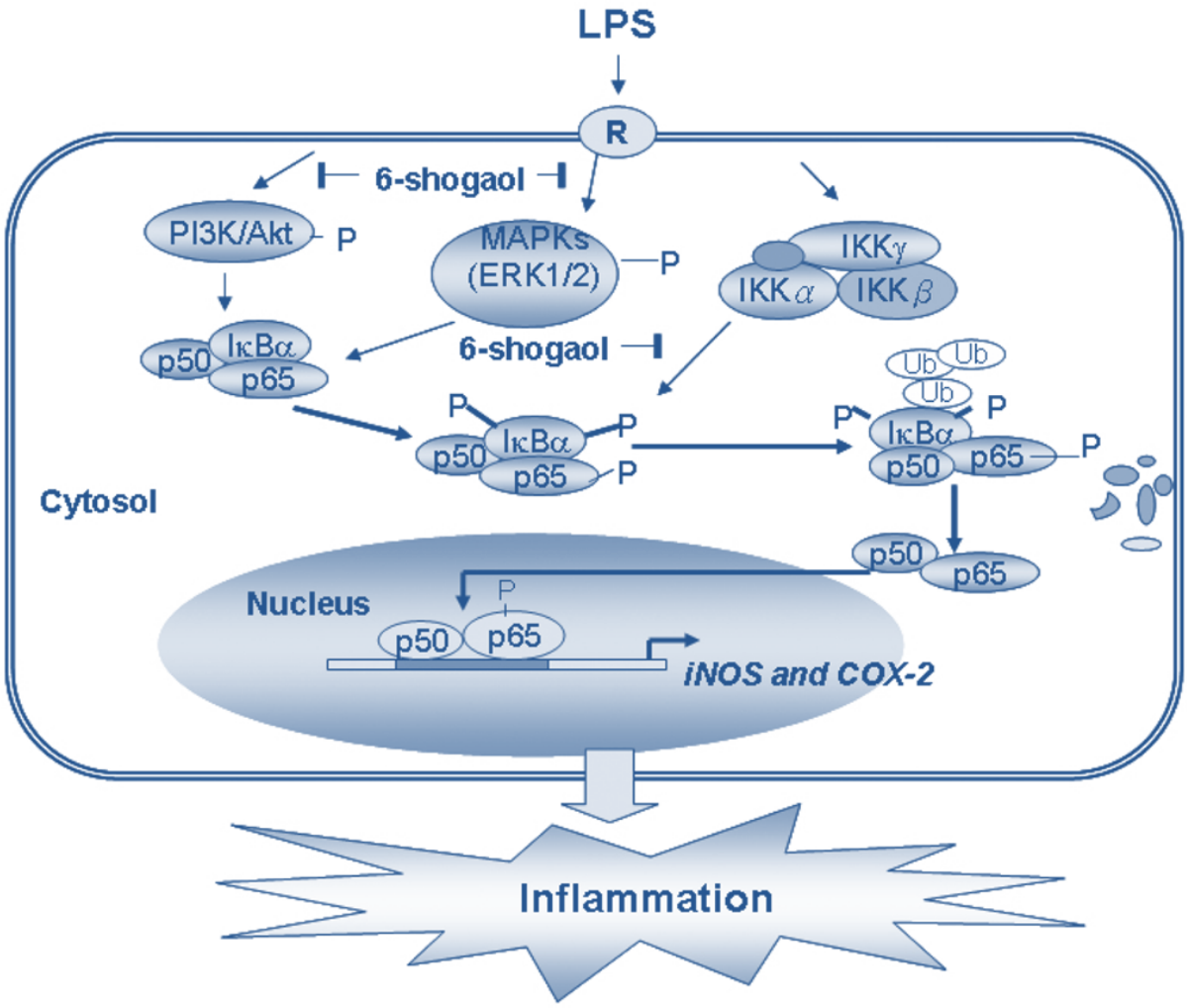


Figure 1.5 *A schematic representation of suppression of LPS induced activation of iNOS and COX-2 expression by 6-shogaol in rat liver macrophages (Pan et al., 2008).*

1.7.3.6. Antitumor effect of ginger

Ginger is listed among the herbs possessing the highest antitumor activities. The observed evidence from in vitro experiments warrants further investigations into the potential anti-tumor activity of ginger. This anti-tumor activity was shown to be related to its components, [6]-gingerol and [6]-shogaol. Both compounds were shown to block the epidermal growth factor responsible for cell transformation, thus inhibiting cellular proliferation.

Ginger may also produce its antitumor effect by inducing "programmed cell death," also known as apoptosis, in cancer cells (Pan, 2008; Davidsen *et al.*, 2006). Other mechanism of ginger's antitumor protection is its modification to the carcinogen metabolizing enzymes in the liver.

The application of [6]-gingerol to the shaven backs of mice prior to applying cancer-promoting agents significantly inhibited skin cancer formation.

[6]-Gingerol also specifically binds with LTA_4H. LTA_4H is identified as a possible molecular target for [6]-gingerol. Accumulating evidence supports a functional role for LTA_4H in cancer development, and therefore targeting LTA_4H is regarded as a useful strategy in chemoprevention and cancer therapy (Flynn *et al.*, 1986). It is shown that, [6]-gingerol directly binds with LTA_4H, and inhibits LTA_4H activity and suppresses the growth of colon cancer cells. [6]-Gingerol suppresses tumor growth by inhibiting LTA_4H activity ((Flynn *et al*, 1986; Kiuchi *et al*., 1982).

[6]-Gingerol suppresses tumor growth through inhibition of LTB_4 production. Carcinogenesis is a multistep process accompanying molecular alterations that drive the progressive transformation of normal cells into highly malignant derivatives. One of the noticeable characteristics of malignant cancer cells is the ability to survive and grow in the absence of anchorage to an extracellular matrix (Kiuchi et al., 1982). Overall, this evidence strongly indicates that inhibition of LTA_4H activity might be a potential target to prevent colorectal carcinoma promotion and progression.

Indeed, [6]-gingerol was reported to suppress epidermal growth factor–induced neoplastic transformation in mouse epidermal cells. In addition, [6]-gingerol inhibits angiogenesis and metastasis, which suggests a broad anticancer activity of [6]-gingerol mediated by multiple mechanisms in various cancers. Collectively, these findings support the anticancer efficacy of [6]-gingerol through its targeting of LTA_4H for the prevention of cancer progression.

1.7.3.7 Antiinflammatory effect of ginger

Targeted inhibition of COX-2 without affecting the housekeeping enzyme COX-1 expression is recognized as one of the most promising strategies for chemoprevention of inflammation. Several lines of evidence have suggested that inducible form of COX-2, a central enzyme in the prostaglandin (PG) biosynthetic pathway plays an important role in tumorigenesis. Prostaglandins are hormone-like fatty acids that participate in many life functions. Not all prostaglandins are beneficial. Excess levels of prostaglandin E2 (PGE2) can cause inflammation (Flynn *et al*, 1986).

COX-2 inhibiting drugs (such as Celebrex and Vioxx) suppress production of PGE2 and are approved by the food and drug administration (FDA) as effective arthritis therapies. Celebrex and Vioxx function specifically by inhibiting the enzyme cyclooxygenase-2 (COX-2). COX-2 is required to convert arachidonic acid into a destructive fatty-acid called prostaglandin E2 (PGE2).

The drugs Celebrex and Vioxx function as COX-2 inhibitors, but as you can see from the chart below that still leaves the lipo-oxygenase pathway open to produce joint-destroying leukotriene B_4.In experimental studies, the pharmacologically active components of the ginger root (gingerols) have been shown to inhibit both the cyclooxygenase and lipo-oxygenase pathways and the production of prostaglandin E2 and leukotrienes. No significant side effects have been reported using gingerols (Kiuchi *et al.*, 1992; Flynn *et al.*, 1986).

Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase-1 and cyclooxygenase-2. An important extension of this early work was the observation that ginger also suppresses leukotriene biosynthesis by inhibiting 5-lipoxygenase (**Figure 1.6**). This pharmacological property distinguishes ginger from nonsteroidal anti-inflammatory drugs. This discovery preceded the observation that dual inhibitors of cyclooxygenase and 5-lipoxygenase (Flynn *et al.*, 1986) may have a better therapeutic profile.

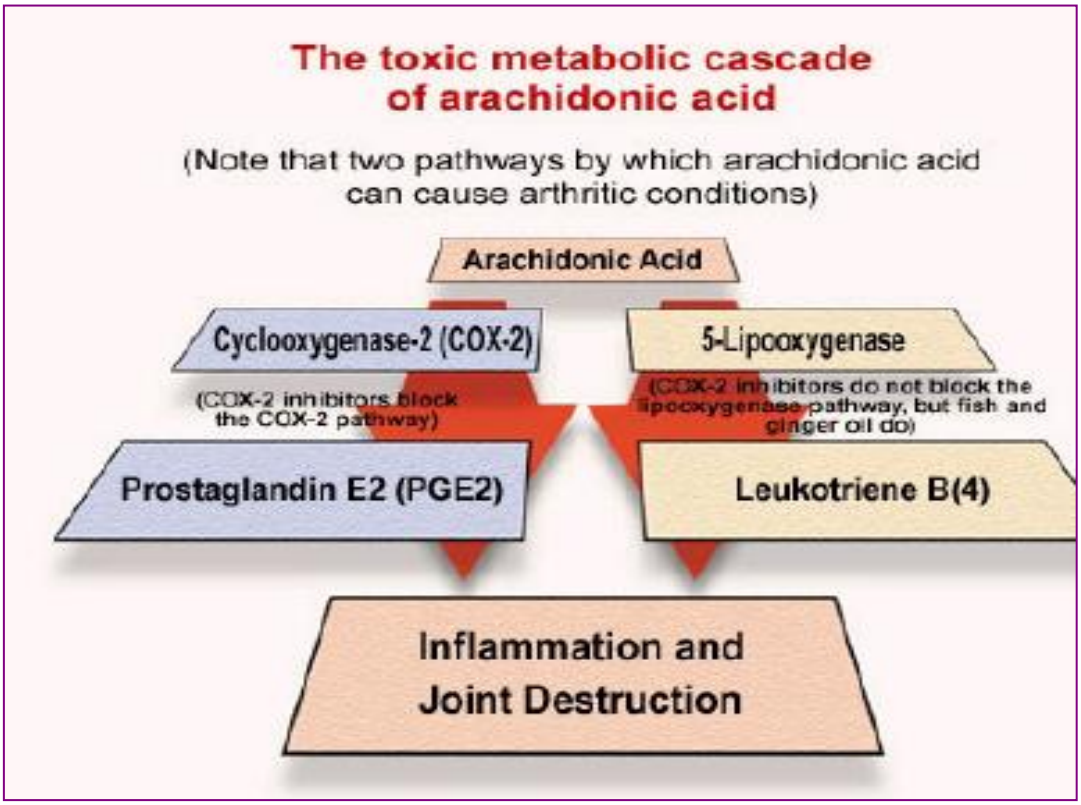


Figure 1.6 *Effect of ginger oil on COX and LOX path ways (Pan et al., 2008).*

The characterization of the pharmacological properties of ginger entered a new phase with the discovery that a ginger extract derived from Zingiber officinale (family Zingiberaceae) inhibits the induction of several genes involved in the inflammatory response. These include genes encoding cytokines, chemokines, and the inducible enzyme cyclooxygenase-2.This discovery provided the first evidence that ginger modulates biochemical pathways activated in chronic inflammation. Identification of the molecular targets of individual ginger constituents provides an opportunity to optimize and standardize ginger products with respect to their effects on specific biomarkers of inflammation.

1.7.3.8 Pharmacokinetics of ginger extracts

Although ginger has been utilized in many studies in both man and animals, there is a relative dearth of information on its disposition in treated subjects. Ginger conjugates began to appear 30 minutes after peritoneal and oral dosing, reaching their T_{max} between 45 to 120 minutes, with elimination half-lives ranging from 75 to 120 minutes at 2.0-g dose.

Intravenous-bolus studies in rats found that free 6-gingerol is rapidly cleared from the plasma with a terminal half-life ranging from 7.23 to 8.5 minutes and that 6-gingerol is not detectable after 30 minutes (Ding *et al.*, 1991). Whereas a single peritoneal and oral dosing of 6-gingerol in rats resulted in the rapid appearance of glucuronide conjugate, it did not reach its maximum concentration until after 12 hours and was detectable for at least 60 hours after ingestion (Nakazawa *et al.*, 2002).

The metabolites seemed to be either glucuronides or sulfates and not mixed conjugates although, once again, 6-gingerol seemed to be an exception with more mixed conjugates seen at lower doses. These results are similar with those observed with peritoneal and oral dosing of 6-gingerol in rats, in which no free 6-gingerol was found at any time point in the urine or bile, but (*S*)-[6]-gingerol-4-*O*-β-glucuronide was present in the bile, and no sulfate conjugates were detectable (Nakazawa *et al*., 2002).

Further intestinal microsomes and hepatic microsomes fortified with uridine diphosphate (UDP)-glucuronyl transferase enzymes only formed glucuronides of 6-gingerol and that uridine guanidine triphosphate (UGT1A1, UGT1A3, and UGT2B7), (these are expressed in both the liver and intestinal mucosa) were responsible for the production of the glucuronides (Asai and Miyazawa, 2000).

Currently, however, it is unclear if gingerols/shogaols are conjugated to glucuronides in the intestinal mucosa, liver or both, and if free or already conjugated gingerols and shogaols reach the liver and are further conjugated with sulfate to form glucuronide/sulfate conjugates there.

Future research will need to be conducted to determine the relative importance of UDP-glucuronyl transferase activity in the liver compared with that in the intestinal mucosa as well as the contribution of liver sulfate enzymes. Intravenous 6–gingerol would bypass being metabolized by the gut bacteria, the intestinal epithelium, or the liver and, thus, in its nonconjugated form that is detectable quickly after injection. Also, it seems that intravenous free 6-gingerol was much more rapidly cleared from the system compared with conjugate metabolites.

In general gingerol, a major constituent of ginger, is rapidly cleared from plasma and elimination by the liver is involved (Naora *et al*., 1992). Gingerol is also a substrate of several UDP-glucuronosyl-transferases, which are major phase 2 metabolic enzymes responsible for the metabolism of several drugs. Gut flora also play a part in the metabolism of gingerol.

1.7.3.9. Toxicity of ginger

The toxicity of ginger is generally considered to be negligible. Oral LD_{50} values in various animals of ginger *oil* exceed 5 gm/Kg (Chen D *et al*., 2007). Ginger has been listed in "**Generally Recognized as Safe"** (GRAS) document of the US FDA, where as in humans a dose of 0.5– 1.0 g of ginger powder ingested 2–3 times a day for periods ranging from 3 months to 2.5 years did not cause any adverse effects.

1.8. Significance of the study

The possible role of crude ginger extract on hepatotoxicity induced by CCl_4 is investigated. The possible mechanism(s) through which ginger exerts its effect is also suggested. A success of exploring the hepatoprotective activity of ginger in various hepatotoxic agents shows the futuristic potential of natural phenolic compounds and is a promising herb that has a full potential of pharmacopeia.

2. HYPOTHESIS

Crude ethanol extract of ginger may ameliorate hepatotoxicity induced by carbon tetrachloride (CCl_4) in Wistar Albino Rats.

2.1 Research Objectives

2.1.1 General objectives

- To investigate the effect of crude ginger extract on hepatotoxicity induced by CCl_4.
- To explore the medicinal use of the plant, (ginger).
- To evaluate the antioxidant properties of crude ginger extract on cells of the liver.

2.1.2. Specific objectives

- To assess the toxic action of CCl_4 in the liver.
- To determine the effect of the extract on liver function enzymes (ALT and AST), and serum total proteins (TP).
- To look in to the efficacy of the extract on the Cholestatic biomarkers (ALP and Bilirubins).
- To assess the correlation between ginger extract and the serum lipid profiles (TC, TG, HDL-C, and LDL-C) level.
- Check whether there is a significant relation between ginger extract and the lipid peroxidation marker (MDA) level.
- To compare and contrast the efficacy of ginger extract and vitamin E in preventing hepatotoxicity-induced by CCl_4.

3. MATERIALS and METHODS

3.1. Plant collection

Ginger rhizome was purchased and brought from welaita zone, bombia wereda which is 43km from the main road (AA to welaita), in side and it is authenticated by the National Herbarium Department Head, Professor Ensermu Kelbesa and got mounted in the herbarium (6th April, 2012 Ethiopia). The fresh succulent rhizome was thoroughly washed and pilled out to remove sand and other impurities, then dried under shade. Dried rhizomes were ground in a grinder with 2 mm diameter mesh and kept in tightly closed container until needed.

3.1.1. Plant Extraction

The dried powdered rhizome (170g) was successively extracted in a Soxlet apparatus using 70% ethanol solvent. Solvent removal was carried out by evaporation using rotary evaporator under reduced pressure at 40^{o}C. From the dried and crushed 530g of ginger rhizome, 160g of ginger crude extract was collected and its percent yield is 30.

3.2. Animals

Adult Male Wistar albino rats (280g ± 90g) were selected for this study. They were obtained from the Animal House of Ethiopian Food and Nutrition Research Institute. The animals were housed in steel mesh cages, (seven per cage) and had a free access to a commercial pellet diet and tap water *ad libitum* for one week before the start of the experiment as an acclimatization period.

3.2.1. Experimental design

28 male Wistar albino rats were used in this study. The body weight of all animals was recorded at the beginning of the experiment. The animals were randomly distributed into seven equally sized four (4) groups according to the following scheme:

Group 1: Received corn oil and served as normal control rats.

Group 2: Received CCl_4 and vitamin E and were considered as standards.

Group 3: Received CCl_4 and ginger extracts and served as treated groups.

Group 4: Rats administered with CCl_4 only and were considered as negative control.

3.2.2. Doses and rout of administration

Administration regimen was for six consecutive weeks. CCl_4 (0.5 mL/kg) suspended in 1mL corn oil was injected intraperitoneally twice weekly. Ginger extract was administrated orally at a dose 200 mg/kg daily (Hamed, 2011). Vitamin E, a reference vegetable drug was intraperitoneally administered at a dose 350 mg/kg daily. Normal control group was orally vehicled with 1mL of corn oil daily.

3.2.3. Sample preparations

Serum sample: Blood from cardiac puncture is collected in clean, dry and gel coated test tube, left 30 minutes to clot and centrifuged at 3000 r.p.m for 15 minutes to separate serum. The separated serum was stored at 80^0C for further determinations of liver function enzymes, lipid profiles, Cholestatic markers (alkaline phosphatase, total and direct bilirubins) and serum total protein.

3.3. Biochemical assays

Except bilirubin and malondialdehyde all parameters were measured by Hitachi autoanalyzer-902 machine.

3.3.1. Antioxidant parameters

3.3.1.1. Determination of Malondialdehyde (MDA) Level

Assay principle

Lipid peroxidation is a well-established mechanism of cellular injury, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including stable reactive carbonyl compounds including MDA. Thus, measurement of MDA has been used as an indicator of lipid peroxidation.

The reaction of MDA as the major thiobarbituric acid (TBA) reactive substances (TBARS) - is the most widely used. Adduct formed during the reaction can be measured both by absorbance and fluorescence.

Antioxidant substances such as the naturally occurring tocopherols and synthetic butylated hydroxyl toluene (BHT/ 2, 6-ditertbutyl-4-methylphenol) reduce the artifactual formation of lipid peroxides during boiling in the presence of atmospheric oxygen and are usually added in the reaction premix before boiling (Wei *et al.*, 2004).

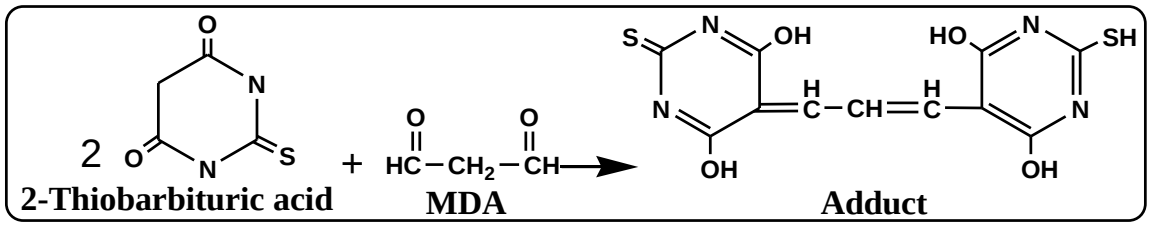


Figure 3.1 *Adduct formations between TBA and MDA*

Procedure

200 µL of serum samples or standards were mixed with 25 µL butylated hydroxyl toluene (BHT) reagent and 200 µL Orthophosphoric acids (0.2 M) in 2 mL Eppindorff's microtubes and vortexed for 10 seconds. 25 µL of TBA reagent were added to the test tubes and vortexed again for the same time.

The reaction mixture was then incubated at 90^0C for 45 minutes in a water bath. The tubes were then put on ice to stop the reaction. After cooling to room temperature, TBARS were extracted once with 500 µL n-butanol.

To facilitate phase separation, 50 µL saturated NaCl solution was added and mixed, and the test tubes were centrifuged at 1200 rpm for 1 minute in an Eppindorff's centrifuge. 250 µL of the upper butanol phase were placed into a flat-bottom 96-well microplate. Absorption was read at 540 nm by small scale ELISA machine. MDA equivalents were quantitatively calculated from calibration curve constructed from absorption vs. concentration of MDA precursor standards.

Table 3.1 *Summarized procedural steps of MDA determination*

Reagents	**Sample(µL)**	**Standard(µL)**	**Reagent Blank(µL)**
Sample/Standard/H_2O	200	200	200
BHT	25	25	25
Orthophosphoric acid; vortex for 10 minutes.	200	200	200
TBA reagent; heating 90 ^{o}C for 45 minutes.	25	25	25
n-butanol; extract.	500	500	500
Saturated NaCl solution, centrifuge.	50	50	50

3.3.2. Serum biomarkers for liver function tests and total protein level

Alanine aminotransferase and Aspartate aminotransferase exist throughout the body.
AST is found primarily in the heart, liver, skeletal muscle, and the kidney. Alanine aminotransferase is found in the liver and the kidneys and, to a lesser extent in the heart and the skeletal muscle.

ALT is exclusively cytoplasmic, where as AST is found both in the mitochondria and cytoplasm. Both enzymes require pyridoxal-5'-phosphate as a cofactor for their activities. Liver diseases particularly infection and drug toxicity are the most important cause of increased serum ALT and AST activity. In most types of liver diseases, ALT is higher than that of AST.

3.3.2.1. Alanine Aminotransferase (ALT) assay

The Alanine Transaminase (ALT) enzymatic assay is based on colorimetric enzymatic assay for the determination of the alanine transaminase enzyme in serum samples. Alanine transaminase (ALT) (also known as alanine aminotransferase or sGPT) is a metabolic enzyme expressed primarily in the liver.
Damage to the liver causes the release of this enzyme into the blood. Elevation of ALT levels is an indication of liver damage and has been associated with liver injury (Paraskevas *et al*., 2010). ALT levels are monitored routinely in patients with liver diseases. ALT is also a very useful tool for preclinical investigation of experimental drug formulations and ALT levels are commonly used to monitor and attenuate the hepatotoxic effects of experimental drugs in rodents (Pratap *et al*., 2010).

Principles of the method

The enzyme ALT catalyzes the transfer of amino group from alanine to 2-oxoglutarate to form glutamate and pyruvate in the presence of pyridoxal-5-phosphate.
The quantity of pyruvate generated is determined by oxidation of nicotine amide adenine dinucleotide, NADH (enzyme coupled assay).

The latter which correlates with serum ALT activity, is determined by continuously monitoring the loss of NADH's absorbance at 340nm.

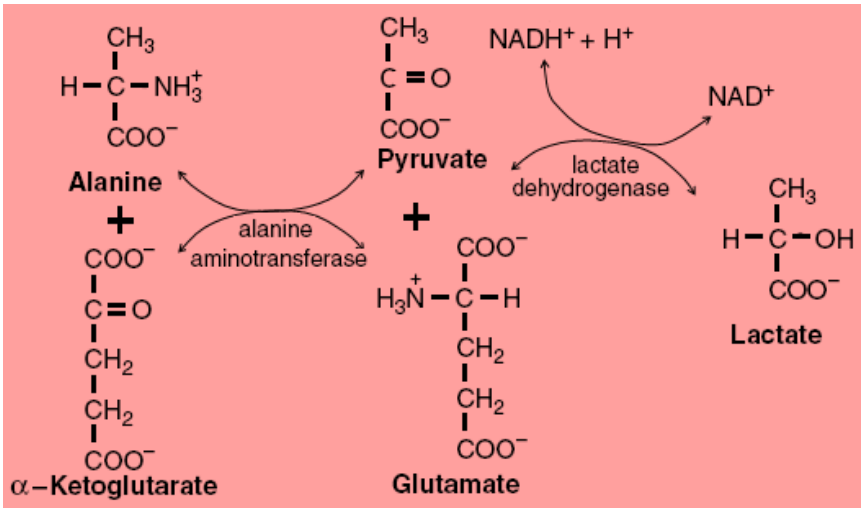


Figure 3.2.*Scheme of ALT reaction principle (Arneson, 2007).*

Procedure

Buffer:	*Substrate:*
	Sodium azide
TRIS buffer (pH 7.5)	α-Ketoglutarate
L-alanine	Sodium azide
LDH	NADH

100µL serum sample was added in to the sample cups and put on to the sample disk which rotates to bring the desired sample cup in to position next to the sample probe for specimen sampling.1000µL buffer and 1000µL substrate were poured in to reagent bottles leveled for ALT and put on to reagent disk and then on the screen menu of the machine ALT as a parameter to be tested was entered.

The sample probe then pipetted sample from the sample disk and transferred to the reaction disk where there is a stirring paddle. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it in to reaction disk which is a large rotatable disk holding reusable cuvettes.

The cuvettes are immersed in to reaction water bath where they were incubated at 37^0C for 5 minutes, and then the reaction disk rotated the cells to all reaction stations including the photometer light path. When the light passed through the cuvettes, absorbance of the sample was measured at 340nm.

3.3.2.2. Aspartate aminotransferase (AST) Assay

The Aspartate Transaminase (AST) enzymatic assay is based on colorimetric enzymatic assay for the determination of the aspartate transaminase enzyme in serum samples. (AST) also known as aspartate aminotransferase or serum glutamate oxaloacetate transaminase (sGOT) which is a metabolic enzyme expressed primarily in the liver.

Elevation of AST levels is an indication of liver damage and has been associated with liver injury (Paraskevas *et al.,* 2010). AST levels are monitored routinely in patients with liver diseases. AST is also a very useful tool for preclinical investigation of experimental drug formulations and AST levels are commonly used to monitor and attenuate the hepatotoxic effects of experimental drugs in rodents (Pratap *et al.,* 2010).

Principles of the method

Aspartate aminotransferase (AST) formerly called (sGOT) catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxaloacetate. The oxaloacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH as illustrated in (**figure 3.3**).
The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of AST present in the sample.

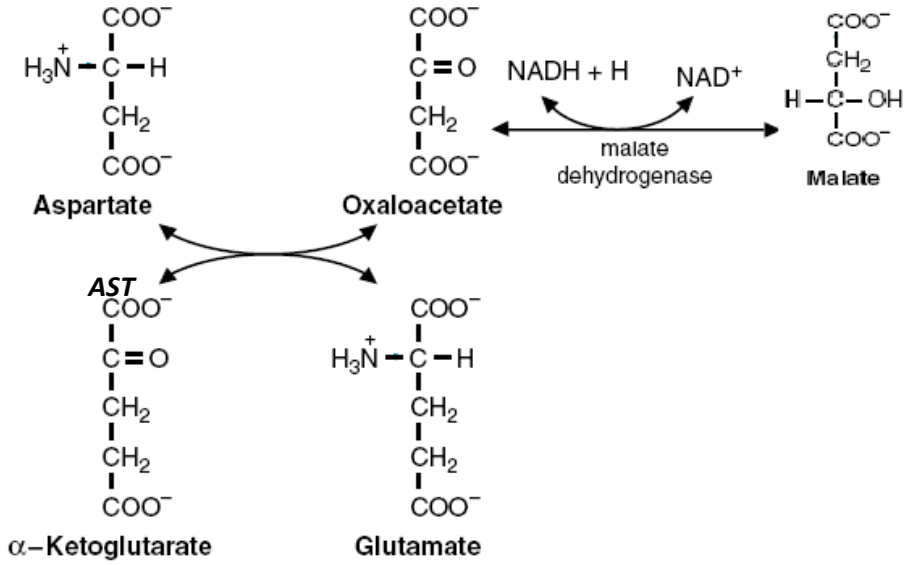


Figure 3.3 *Scheme of AST reaction principles (Arneson, 2007).*

Procedures

Buffer	Substrate
2-oxoglutarate	Tris-buffer (pH 7.9)
NADH	L-aspartate
Sodium azide	LDH, MDH, and sodium azide

100µL serum sample was added in to the sample cups and put on to the sample disk which rotates to bring the desired sample cup in to position next to the sample probe for specimen sampling.1000µL buffer and 1000µL substrate were poured in to reagent bottles leveled for AST and put on to reagent disk and then on the screen menu of the machine AST as a parameter to be tested was entered.

The sample probe then pipetted sample from the sample disk and transferred to the reaction disk where there is a stirring paddle. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it in to reaction disk which is a large rotatable disk holding reusable cuvettes.

The cuvettes are immersed in to reaction water bath where they were incubated at 37^0C for 5 minutes, and then the reaction disk rotated the cells to all reaction stations including the photometer light path. When the light passed through the cuvettes, absorbance of the sample was measured at 340nm.

3.3.2.3. Method of determination of total proteins by Refractometry

Principles of the method

Refractometry is a rapid analytical method that is used to determine serum proteins when a very small volume of serum is needed. The velocity of light is changed as it passes the boundary between two transparent layers (e.g. air and water).When a solute is added to the water, the refractive index at 20^0 of 1.33 for water is increased by an amount proportional to the concentration of the solute in dilute solutions.

The refractive index is also temperature dependent, but some refractometers incorporate a built in temperature correction and an assumption is made that constituents like electrolytes, urea, and glucose are present in nearly the same concentration as in the calibrating serum used to establish the built in scale in the refractometer (Lines and Raines, 1970) .

Procedure

Total proteins are the major masses of the dissolved solids in blood plasma and are commonly measured by a hand held refractometer.

A drop (50µL) of serum is placed by capillary action between a cover glass and the prism. The refractometer is held so that light is refracted through the serum layer. The refracted rays caused part of the field of view to be light, producing a point at which there is a sharp line between light and dark. The number of g/dL at this line on the internal scale was read.

3.4. Cholestatic indices

3.4.1. Alkaline phosphatase assay

Principles of the method

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in an alkaline environment, resulting in the formation of an organic radical and inorganic phosphate. In mammals, this enzyme is found mainly in the liver and bones.

Marked increase in serum ALP levels, a disease known as hyperalkalinephosphatasemia, has been associated with malignant biliary obstruction, primary biliary cirrhosis, hepatic lymphoma and sarcoidosis. ALP can hydrolyze p-Nitrophenylphosphate (pNPP) releasing p-Nitrophenol (pNP) and Phosphate with a yellow colored product. The rate of pNP(quinonid form) release, which correlates with serum alkaline phosphatase activity, is determined by continuous monitoring of the increase in absorbance at 405 nm.

Reaction principle

$$p\text{–}Nitrophenylphosphate + H_2O \xrightarrow{ALP\ Mg^{+2}} p\text{–}nitrophenyl + phosphate\ ion$$

The rate of the reaction is directly proportional to the enzyme activity.

Procedures

Buffers	Diethanolamine buffer (pH 10)	*Substrate*
	Magnesium chloride	p-Nitrophenyl phosphate

20µL serum sample was added in to the sample cups and put on to the sample disk which rotates to bring the desired sample cup in to position next to the sample probe for specimen sampling.

1000µL buffer and 1000µL substrate were poured in to reagent bottles leveled for ALP and put on to reagent disk and then on the screen menu of the machine, ALP as a parameter to be tested was entered.
The sample probe then pipetted sample from the sample disk and transferred to the reaction disk where there is a stirring paddle. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it in to reaction disk which is a large rotatable disk holding reusable cuvettes.

The cuvettes are immersed in to reaction water bath where they were incubated at 37^0C for 5 minutes, and then the reaction disk rotated the cells to all reaction stations including the photometer light path. When the light passed through the cuvettes, absorbance of the sample was measured at 405nm.

3.4.2. Colorimetric assay of Bilirubin (JANDRASSSIC-GRUFF) principle

The canalicular transport mechanism for excretion of bilirubin conjugates is especially sensitive to injury. Accordingly, in parenchymal liver disease or mechanical bile duct obstruction, bilirubin conjugates within the hepatocyte or biliary tract may reflux into the blood stream, resulting in a mixed or, less often, a purely conjugated hyperbilirubinemia.

Unconjugated bilirubin is not excreted in urine regardless of the level of its plasma concentration because its binding to albumin is too tight for effective glomerular filtration and it is not secreted by the tubules.
Conjugated bilirubin, which is normally loosely bound to albumin, is readily filtered at the glomerulus; even modest degrees of conjugated hyperbilirubinemia result in bilirubinuria, which is always a pathologic finding.

Clinical laboratories typically quantify plasma bilirubin by a reaction in which bilirubin is cleaved by a diazo reagent, such as diazotized sulfanilic acid, to azodipyrroles that are quantitated spectrophotometrically (Jandrasssic, 1981).
Bilirubin conjugates react rapidly (“prompt” or “direct”- reacting bilirubin). Unconjugated bilirubin reacts slowly because the site of attack by the diazo reagent is protected by internal hydrogen bonding. Accordingly, accurate measurement of the total plasma bilirubin concentration requires addition of an accelerator, such as methanol or urea, to disrupt this internal bonding and to ensure complete reaction of any unconjugated bilirubin.

Total bilirubin is coupled with a diazonium sulphanilic acid to yield the corresponding azobilirubin.The absorbance of this blue green dye at 600nm is directly proportional to the total concentration of bilirubin. The values measured in the absence of solubilizers like methanol is called direct bilirubin where as in their presence is total bilirubin.

Procedure

Table 3.4 Assay procedures of bilirubin determination.

	Direct bilirubin		**Total bilirubin**	
Reagents	Sample blank(µL)	Sample test(µL)	Sample blank(µL)	Sample test(µL)
Serum sample	500	500	500	500
Distilled water	500	500		
Methanol			500	500
1.5%HCl	100		100	
Diazo(diazotized sulfanilic acid)		100		100

Incubated at 37^0C for 10 minutes and then read by the semi-automated humanlyzer3000 machine.

3.5. Serum lipid profiles

Clinical chemistry laboratories offer many tests for lipid disorders. One of the most common tests is the lipid profile. This panel of tests includes measurements of Triacylglycerol, total cholesterol and cholesterol in the form of low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). The results of testing these lipids provide measures of risk for coronary artery disease (Arneson and Brickell, 2007). Although the concentration of cholesterol in blood is dependent on many factors such as genetics, age, sex, diet, and physical activity, total cholesterol measurement is used clinically to monitor disease. In addition to its role as a risk factor for coronary artery disease, increased cholesterol concentration may be the result of liver disease, renal disease, or diabetes.

Animals were fasted for 12-hours before blood samples were obtained. Blood was collected by cardiac puncture from each rat into a gel coated centrifuge tubes. The blood samples were centrifuged in a Denley BS400 centrifuge (England) at 5000 r.p.m for 5-minutes. The supernatant (serum) collected was assayed for the serum levels of TG, TC and HDL-C, while (LDL-C) was calculated using the (Friedewald *et al*., 1972) equation.

$$LDL\,cholesterol = Total\,cholesterol - \left(\frac{Triglycerides}{5} - HDL\,cholesterol \right)$$

3.5.1. Serum HDL-cholesterol determination

Principles of the method

The very low and the low density lipoproteins from serum are precipitated by phosphotungstate in the presence of magnesium chloride (Naito *et al.,* 1984*)*. After removal by centrifugation the clear supernatant is used for the determination of HDL-cholesterol.

Procedures

A 200µL serum sample was pipetted in to a centrifuge tube and then 100µL of the precipitating reagent containing phosphotungisic acid and magnesium chloride was added in to the tube that contained serum samples. After thorough mixing it was allowed to stand for 10 minutes at room temperature and then centrifuged at 5000 r.p.m for 5 minutes. Supernatant was collected and the HDL-cholesterol was detected using Roche/Hitachi 902 autoanalyzer, similar to TC and TG.

3.5.2. Colorimetric determination of total cholesterol

Principles of the method

Cholesterol is determined after enzymatic hydrolysis by cholesterol esterase (CHE) and oxidation (Rischlau *et al.*, 1974). The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol peroxidase enzyme (POD). This is a three step reaction that allows for quantitation of cholesterol esters and free cholesterol.

Reaction principles

$$Cholesterol\ ester + H_2O \xrightarrow{CHE} Cholesterol + Fatty\ acids$$

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

$$2H_20_2 + 4 - a\min ophenazone + phenol \xrightarrow{POD} quinone\min e + 4H_2O + phenazone + phenol$$

The enzymatic assay of cholesterol shows more specificity than the other colorimetric assays and this is because of cholesterol oxidase (CHO) which is specific for sterols with a 3-beta hydroxyl and a double bond in the C4-5 or the C5-6 position.

Procedures

Buffer	*Reagents*
Phosphate buffer (pH 6.5)	4-Aminophenazone, phenol, and phenol peroxidase Cholesterol oxidase and Sodium azide

10µL serum sample was added in to the sample cups and put on to the sample disk which rotates to bring the desired sample cup in to position next to the sample probe for specimen sampling.

1000µL buffer and 1000µL substrate were also pipetted in to reagent bottles leveled for TC and put on to reagent disk and then on the screen menu of the machine TC as a parameter to be tested were entered.

The sample probe then pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it in to reaction disk which is a large rotatable disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents.

The cuvettes are immersed in to reaction water bath where they were incubated at 37^0C for 5 minutes, and then the reaction disk rotated the cells to all reaction stations including the photometer light path. When the light passed through the cuvettes, absorbance of the sample was measured at 500nm.

3.5.3. Serum Triacylglycerol assay

Principles of the method

Sample triglycerides incubated with lipoprotein lipase (LPL), liberate glycerol and free fatty acids (Buccolo *et al.,* 1973). Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-phosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetonphosphate (DAP) and hydrogen peroxide (H_2O_2).

In the last reaction hydrogen peroxide reacts with 4-aminophenazone (4-AP) and p-chlorophenol in the presence of peroxidase (POD) to give a red color.

Reaction principles

$$Triglycerides + H_2O \xrightarrow{LPL} Glycerol + Free\ fatty\ acids$$

$$Glycerol + ATP \xrightarrow{GK} Glycerol - 3 - Phosphate + ADP$$

$$Glycerol - 3 - phosphate + O_2 \xrightarrow{GPO} DAP + H_2O_2$$

$$H_2O_2 + 4 - AP + P - chlorophenol \xrightarrow{POD} Quinone + H_2O$$

The intensity of the color is directly proportional to the triglycerides concentration in the sample.

Procedure

10µL serum sample was added in to the sample cups and put on to the sample disk which rotates to bring the desired sample cup in to position next to the sample probe for specimen sampling.1000µL buffer and 1000µL substrate were also pipetted in to reagent bottles leveled for TG and put on to reagent disk and then on the screen menu of the machine TG as a parameter to be tested was entered.

The sample probe then pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it in to reaction disk which is a large rotatable disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents.

The cuvettes are immersed in to reaction water bath where they were incubated at 37^0C for 5 minutes, and then the reaction disk rotated the cells to all reaction stations including the photometer light path. When the light passed through the cuvettes, absorbance of the sample was measured at 505nm.

3.6. Study area

The study is conducted in Addis Ababa University, School of Medicine (Tikur Anbessa Specialized Teaching Hospital) particularly in Biochemistry Department.

3.7. Statistical Analysis and Calculations

Statistical analysis is carried out by one-way and two-way analysis of variance (ANOVA), using graph pad prism 5. All data are expressed as Mean ± SEM of seven rats in each group.
Significant difference between groups was at $p< 0.05$.
% change = control mean - treated mean / control mean x 100.
% improvement = injured mean - treated mean / control mean x 100.

4. RESULTS

4.1. Effect of *Zingiber officinale* on hepatic antioxidant levels

CCl_4 treated group showed significant increase in MDA level by 18% when compared to normal group. Treatment with ethanol extract of the plant improved MDA levels by 78% and treatment with vitamin E recorded improvement in MDA level by 49% compared to negative control. MDA level in treated group declined more than the standards and this shows that ginger is more effective antioxidant than vitamin E.

Table 4.1 Effect of *Zingiber officinale* on Malondialdehyde (MDA) level

Groups	**MDA(µmol/L)**
Normal control	3.17±0.58
Standards	2.18 ± 0.86^{db}*
Treated groups	1.27 ± 0.78^{dc}**
Negative control	3.75 ± 0.14^{d}

Statistical analysis is carried out using one-way analysis of variance (ANOVA).

Comparisons were made between:

"a" normal control vs. CCl_4 group, "b" standard group vs. CCl_4 group, "c" Treated group vs. CCl_4 group, and "d" none significant difference between the groups.

**= shows significant difference between negative control and all the rest of the groups, *= p<0.05, **= p<0.01, ***= p<0.001*

4.2. Effect of *Zingiber officinale* on Serum lipid profiles (TC, TG, HDL-C and LDL-C)

CCl_4 group showed significant increase in TC, TG, and LDL-C levels by 36%, 38%, and 105%, respectively compared to normal control. However, serum HDL-C was decreased by 16%. After the extract treatment, TC, TG, and LDL-C levels significantly decreased by 34%, 45%, and 102%, respectively when compared to negative control, while HDL-C showed a significant increase by 27.4%. In vitamin E treated group TC, TG, and LDL-c showed significant decrease by 27.9%, 44%, and 82%, respectively, but HDL-C showed no significant increase when compared with negative control. No significant difference in lipid profiles was shown between normal control and treated groups.

Table 4.2 Effect of *Zingiber officinale* extracts on Serum Lipid Profiles (mg/dL) in CCl4 treated rats.

Groups	TC(mg/dL)	TG(mg/dL)	HDL-c(mg/dL)	LDL-c(mg/dL)
Normal control	154.8±6.02	119.8±4.49	70±4.52	57.32±2.41
Standards	168.2±5.59^{db***}	112.4±7.6 ^{db***}	75.6±1.99^{d}	70.24±4.75^{db***}
Treated groups	158.6±5.32^{dc***}	110.54±3.95^{dc***}	79.8±2.58^{dc*}	58.7±4.63^{dc***}
Negative control	211.2±4.59^{a*}	165.2±4.85^{a***}	60.6±4.34^{a*}	117.6±4.56^{a***}

Statistical analysis is carried out using two-way analysis of variance (ANOVA).
Comparisons were made between:

"a" normal control vs. CCl_4 group, "b" standard group vs. CCl_4 group, "c" Treated group vs. CCl_4 group, and "d" none significant difference between the groups.

**= shows significant difference between negative control and all the rest of the groups, *= p<0.05, **= p<0.01, ***= p<0.001*

4.3. Potency of *Zingiber officinale* in improving liver function enzymes and serum protein

CCl_4 intoxicated group showed significant increase in AST and ALT levels by 78.6% and 79% respectively compared to normal controls. However, serum total protein was decreased by 29%. The observed changes in liver function enzymes showed that ethanol extract recorded the most improvement percentages. AST and ALT were ameliorated by 59.6% and 64.7% respectively, while serum protein was improved by 27% compared to negative control.
Vitamin E decreased AST and ALT levels by 72.6%, and 71.2% respectively, but total protein levels were shown to increase by 26.7% when compared to negative control.

Table 4.3 Effect of *Zingiber officinale* extracts on liver function enzymes and total protein level in serum of CCl_4 treated rats.

Groups	ALT(u/L)	AST(u/L)	TP(g/dL)
Normal control	203.8±24.43	244.6±9.93	8.6±0.46
Standards	220.8±19.4^{db}***	260.2±12.76^{db}***	8.44±0.35^{db}**
Treated groups	233.28±17.64^{dc}**	292.24±25.7^{dc}***	8.46±0.39^{dc}**
Negative control	365.2±25.72^{a}**	438±45.38^{d}	6.14±0.50^{a}*

Statistical analysis is carried out using one-way analysis of variance (ANOVA).
Comparisons were made between:

"a" normal control vs. CCl_4 group, "b" standard group vs. CCl_4 group, "c" Treated group vs. CCl_4 group, and "d" none significant difference between the groups.

**= shows significant difference between negative control and all the rest of the groups, *= p<0.05, **= p<0.01, ***= p<0.001*

4.4. Efficacy of *Zingiber officinale* on serum Cholestatic markers

As compared to normal healthy rats, CCl_4 group recorded significant increase in ALP, direct and total bilirubins by 55.7%, 46.6% and 157%, respectively when compared to normal control. Treatment with ethanol extract of ginger recorded the highest improving percent in ALP, direct and total bilirubins by 57%, 173% and 107%, respectively compared to negative control.

ALP, direct and total bilirubins recorded percentages improvement reached to 60%, 180% and 109%, respectively after vitamin E treatment when compared to negative control, but no significant difference were shown among normal, treated and standard groups.

Table 4.4 Effect of *Zingiber officinale* extracts on serum Cholestatic markers in CCl4 treated rats.

Groups	**ALP(u/L)**	**DB(mg/dL)**	**TB(mg/dL)**
Normal control	288.20±26.87	0.26±0.06	0.82±0.31
Standards	295.4±34.46^{db}**	0.32±0.04^{db}*	0.78±0.08^{db}***
Treated groups	305±5.59^{dc}**	0.34±0.06^{dc}*	0.79±0.08^{dc}***
Negative control	468±39.07^{a}***	0.79±0.06^{a}**	1.67±0.16^{d}

Statistical analysis is carried out using one-way analysis of variance (ANOVA) for ALP and two-way analysis of variance (ANOVA) for bilirubins.

Comparisons were made between:

"a"- normal control vs. CCl_4 group, "b"- standard group vs. CCl_4 group, "c"- treated group vs. CCl_4 group, and "d"- none significant difference between the groups.

**= shows significant difference between negative control and all the rest of the groups, *= p<0.05, **= p<0.01, ***= p<0.001*

4.5. Effect of *Zingiber officinale* on body weights of CCl_4 treated rats

CCl_4 intoxicated rats showed significant (18.3%) decrease in body weight by compared to normal control, but ginger extract treated rats showed significant (14%) improvement in body weight by and vitamin E treated groups improved their body weight by 1.3% when it is compared to negative control.

Table 4.5 Effect of *Zingiber officinale* extracts on body weights of ccl_4 treated rats.

Groups	**Weight Before(g)**	**Weight After(g)**
Normal Control	152.8±4.73	183.4±5.46
Standards	$233.8±10.65^{db***}$	$236±8.39^{db***}$
Treated groups	$215±5.51^{dc*}$	$245.2±6.74^{dc***}$
Negative control	$186.2±6.16^{a**}$	$155.6±10.994^{d}$

Statistical analysis is carried out using two-way analysis of variance (ANOVA).

Comparisons were made between:

"a" - normal control vs. CCl_4 group, "b"- standard group vs. CCl_4 group, "c"- treated group vs. CCl_4 group, and "d"- none significant difference between the groups.

**= shows significant difference between negative control and all the rest of the groups, *= p<0.05, **= p<0.01, ***= p<0.001*

5. Discussion

CCl_4 is considered as one of the most commonly used hepatotoxins in the experimental study of liver diseases. The reactive metabolite trichloromethyl radical ($\cdot CCl_3$-) has been formed from the metabolic conversion of CCl_4 by cytochrome P-450. This reactive metabolite initiates the peroxidation of membrane polyunsaturated fatty acids (PUFA), generates PUFA radicals, covalently binds to membrane lipids and proteins and generates ROS (Gowri *et al.*, 2008).

Evidence suggests that various enzymatic and non-enzymatic systems have been developed by the cell to attenuate ROS. However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient. This may cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. Therefore, ROS affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH, decreases the activity of SOD and this leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally results in cell death (Opoku *et al*., 2007). Decrease in GSH activity might be due to decrease availability of GSH resulted during the enhanced lipid peroxidation processes.

Hepatoprotective studies showed that plants have active ingredients that are capable of free radical scavenging in living systems (Mitra *et al.,* 1995).Flavonoids, sterols, triterpenes and alkaloids as antioxidative compounds are rich in most plants (Opoku *et al.*, 2007). In the present study and in accordance with (Opoku *et al.*, 2007), high content of these compounds was recorded in the ethanol extract of ginger suggesting its potential role as antioxidative agent.

Treatment with ginger extracts normalized the antioxidant levels through their content of flavonoids that have the ability to scavenge free radicals and this was seen when ginger extracts attenuated the increased level of serum enzymes and caused a subsequent recovery towards normalization. This gave an additional support that ginger extracts are able to condition the hepatocyte, accelerate regeneration of parenchyma cells, and protect against membrane fragility and decrease leakage of the enzymes into circulation. Vitamin E as an antioxidant complex has the ability to quench free radicals elevation, chelates metal ions, inhibits lipid peroxidation and prevents liver glutathione depletion (Mansour *et al*, 2006).

The level of lipids peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, significant ($p<0.05$) elevation of lipid peroxidation in the liver of rats treated with CCl_4 was observed.

The increase in MDA levels in the liver suggests enhanced lipid peroxidation leading to tissue damage, lipid peroxidative degradation of biomembranes and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals due to hepatotoxicity caused by CCl_4 and this agrees with (Kikuzaki, 1993) who said that CCl_4 administration leads to parallel increase in MDA and collagen, and subsequent decrease in SOD.

The hepatic lipid peroxidation which was enhanced in the CCl_4 treated group was improved by ginger extract, where there was a reduction in malondialdehyde (MDA) level by 78% ($p<0.01$), but vitamin E decreased MDA level only by 49%($p<0.05$) which is less potent than ginger extract (**Table 4.1)**, so that it is possible to say ginger protection was effective to render hepatoprotection as evident from decreased hepatic MDA level when compared to CCl_4 treated group and vitamin E and this is because Zingerone inhibits liver microsomal lipid peroxidation.

Results obtained from this study demonstrate that CCl_4 intoxication caused hepatocellular damage represented by the marked elevation in serum ALT (79%) and AST (77.9%) activities, compared to the normal control (**Table 4.3**). These enzymes are considered the most sensitive markers of liver injury as they are found in the cytoplasm and mitochondria of liver cells, thus damage of these cells lead to their rapid leakage of the above enzymes into the blood circulation (Opoku *et al*., 2007).

As data in (**Table 4.3)** showed, significant elevation of ALT and AST after CCl_4 intoxication was in agreement with those reported studies of (Opoku *et al*., 2007) and (Gowri *et al*., 2008),who explained that serum enzymes elevation indicates increase in hepatic cell membrane fluidity that led to enzyme release into circulation. Meanwhile, the significant decrease in serum AST (64.7%, $p<0.001$) and ALT (59.6%, $p<0.01$) activities in ginger+ CCl_4 group elucidate the hepatoprotective effect of ginger extract and this is in the same line with(Albano,1987) who declared that the hepatoprotective effect of ginger aqueous infusion when given orally to the rats was evident by significant reduction in ALT($p<0.05$), AST($p<0.01$), ALP($p<0.05$) and total bilirubin($p<0.05$) in rat groups receiving ginger before giving paracetamol when compared to the rats group receiving paracetamol only.

These results agree with those obtained by (Alnaqeeb *et al*., 2003) who found that the administration of aqueous extract of ginger to rat orally and intraperitoneally at two different levels of doses decreased some serum enzymes activity such as AST($p<0.001$) and ALT($p<0.05$) significantly.

Under condition of severe oxidative stress, free radical generation leads to protein modification. Protein may be damaged directly by specific interaction of oxidants or free radicals with particularly susceptible amino acids (Narasimhana and Ponnaian, 2006).
In addition, (Romero *et al*., 1998) showed that CCl_4 intoxication induced changes in the process of protein synthesis. Hence, decrease in total protein content can be deemed as a useful index of the severity of cellular dysfunction in liver diseases after CCl_4 intoxication as clearly shown in this study.
Stimulation of protein synthesis by 27% ($p<0.01$), (**Table 4.3**) after ginger extract treatment has been advanced as a contributory self healing mechanism, which accelerates liver regeneration process (Sharma and Shukla *et al.*, 2010).Though there is a small difference in percent of reduction, ginger ameliorated liver enzymes and total proteins in the same potential and mode of action to vitamin E.

Hyperbilirubinemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate (Meshkibaf, 2006). ALP and bilirubin were observed to increase in the present study (**Table 4.4**). This was in agreement with (Reyes *et al*., 2007), who recorded significant increase in cholestasis biomarker after intoxication of rats with CCl_4. Ginger was shown to have the same potency as vitamin E in decreasing cholestatic markers.

Leonard *et al*., (1984) confirmed bilirubin as indicator of bile duct lesions. Depletion of elevated bilirubin (DB, 56.6% ($p<0.05$) and TB, 173% ($p<0.001$)) level together with the suppression of the activity of ALP by 56.6% ($p<0.01$) in the serum of rats treated with ethanolic rhizome extracts of ginger suggests the possibility of phytotherapeutic option being able to stabilize biliary dysfunction of rat liver during chronic injury with CCl_4. This increase in bilirubin concentration in the serum indicate that bile is not being excreted and/or that too much hemoglobin is being destroyed and/or that the liver is not actively treating the hemoglobin it is receiving and could therefore lead to jaundice.

Regulatory effect of ginger extracts and vitamin E on hepatic marker enzymes is documented in this study as free radicals scavengers that could in turn normalize microsomes, lysosomes, mitochondria and plasma membranes permeability and integrity which lead to restore the hepatic enzymes to its normal levels.

In the present study, CCl_4 intoxicated rats was shown to have maximum increment in their total cholesterol, but those followed by the extracts of ginger were shown to have significantly decreased serum total cholesterol level by 34% ($p<0.001$), and this was in agreement with (Murugaiah *et al*., 1999), ginger reduced serum and tissue cholesterol ($p<0.01$). The cholesterol-lowering effects of ginger root in ginger treated rats is related to an increased excretion of cholesterol, neutral sterols, bile acid and an increase in hepatic bile acid content (Visavadiya and Narasimhasharya, 2006). Vitamin E decreased serum total cholesterol level by 28%, but is less effective when compared to ginger.

In this context, the presence of phytosterols, saponins and fiber in Ginger root could be important in cholesterol elimination and an increase in hepatic bile acid content. Phytosterols are reported to displace intestinal cholesterol and reduce cholesterol absorption from intestine. Saponins on the other hand, are capable of precipitating cholesterol from micelles and interfere with enterohepatic circulation of bile acids making it unavailable for intestinal absorption and result in depletion of hepatic cholesterol pools.

The accelerated fecal excretion of cholesterol in these animals could also be a response to the relatively higher fiber content of the root that increases the activity of plasma lecithin cholesterol acyltransferase (LCAT), enhances hepatic bile acids synthesis and increases degradation of cholesterol to fecal bile acids and neutral sterols. This view is in accordance with an earlier report suggesting that the cholesterol lowering effect of fiber content.

In ginger treated groups triacylglyceride was significantly reduced by 45% ($p<0.001$), indicating the hypotriglyceridaemic effect of the extract and this is because both dietary fibers and saponins are known to lower TG by decreasing hepatic lipogenesis and inhibiting pancreatic lipase activity respectively (Visavadiya and Narasimhasharya, 2006), and this is similar to the findings of (Murugaiah *et al*., 1999) who revealed that ginger reduced serum and tissue triglycerides ($p<0.05$).

The result also showed that ginger has an effect on low density lipoprotein cholesterol (LDL). CCl_4 intoxicated rats followed by ginger extract showed significant decrease in serum LDL cholesterol by 105% ($p<0.001$) and this was in agreement with (Murugaiah *et al*., 1999)who claimed that ginger reduced serum and tissue LDL cholesterol($p<0.001$).

A significant decline in plasma LDL-cholesterol in these groups could be correlated with the fiber and saponin content of the plant as both fibers and saponins enhance the hepatic LDL-receptor levels, increase hepatic uptake of LDL-cholesterol and aid its catabolism to bile acids and this is in accordance with (Diederchsen,1996) who reported that omega 3 fatty acids and soluble fibers play a role in decreasing LDL-level by 46% ($p<0.01$), because omega 3 fatty acids incorporate into atherosclerotic plaques and rupture it and it is also reported that omega 3 fatty acids increased LDL-c receptors that enhance LDL-c entry into cells. On the other hand ginger showed a significant increment in HDL-C by 27.4% ($p<0.05$). This high level of plasma HDL-cholesterol in ginger treated animals indicates its efficacy in elevating HDL-cholesterol levels this is because omega-3-fatty acids content of the plant inhibits Apo D activity which is responsible for the transferring of cholesteryl ester (CE) into VLDL. (Murugaiah *et al*., 1999) suggested that mixing of ginger to rat's food increased HDL-c level significantly ($p<0.05$).

The observed significant ($p<0.05$) increase in HDL-cholesterol concentration upon the administration of the extract indicates that the extract have HDL-cholesterol boosting effect. Hence large amount of cholesterol is removed from the circulation. These findings are in agreement with previous studies as (Bhandari *et al*., 2005) revealed that ethanolic extract of ginger produced significant ($p<0.05$) decrease in serum total cholesterol and triglycerides levels and increased HDL-cholesterol level as compared to negative control rats and the extract exhibit a significant lipid lowering activity and protect the tissues from lipid peroxidation, but no significant increase of HDL-cholesterol was shown in vitamin E treated group.

Fuhrman *et al*., (2000) revealed that ethanolic extract of ginger reduced plasma cholesterol and inhibited LDL oxidation in atherosclerotic, apolipoprotein E-deficient mice. (Srinivasan and Sambaiah, 1991) reported that feeding rats with ginger significantly elevated the activity of hepatic cholesterol 7-alpha-hydroxylase. Furthermore, (Ness *et al*., 1996) reported that the reduction of cellular cholesterol biosynthesis is associated with increased activity of the LDL receptor, which in turn leads to enhanced removal of LDL from plasma, resulting in reduced plasma cholesterol concentration and showing that plant ginger possesses cholesterol-suppressive capacity. In the study of (Bruan and Severson, 1992) it was shown that deficiency of lipoprotein lipase activity may contribute significantly to the elevation of triglycerides in diabetes. Furthermore, (Lopes, 1997) reported that treatment of diabetes with insulin served to lower plasma triglycerides levels by returning lipoprotein lipase levels to normal.
In the present study the decreasing levels of plasma triglycerides following the treatment with ginger extract may be due to the stimulating effect of ginger extract on insulin.

The lipid per-oxidation lowering effect associated with ginger consumption was also demonstrated in apolipoprotein E-deficient mice (i.e., mice that are prone to develop atherosclerosis). Mice that consumed ginger in their drinking water showed a significant reduction in their basal concentration of LDL-associated lipid peroxides. A number of animal studies have shown that ginger lowers cholesterol levels. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic rats (Coppola *et al*., 2007).

Hypercholesterolemia is a major risk factor for atherosclerosis and reduction in plasma cholesterol concentration by drug therapy has reduced cardiovascular incidence (Rhode *et al*., 2007). Consumption of natural nutrients, capable of reducing plasma cholesterol, thus, should also reduce development of atherosclerosis. It is demonstrated that dietary consumption of ginger extract significantly reduced the development of aortic atherosclerotic lesions, along with an impressive reduction in the levels of plasma and LDL cholesterol.

Cholesterol biosynthesis in peritoneal macrophages may represent cholesterol synthesis in the liver, which has a major role in determining lipoprotein level in plasma. Reduced cellular cholesterol biosynthesis is associated with increased activity of the LDL receptor, which in turn leads to enhanced removal of LDL from plasma; resulting in reduced plasma cholesterol concentration in arterial wall macrophages play a major role during early atherogenesis.

Oxidative stress induces macrophage responses such as increased capacity to oxidize LDL, increased Ox-LDL cellular uptake, as well as macrophage lipid peroxidation (Coppola *et al*., 2007), but ginger counteracts the above all this is by increasing HDL which suppress LDL oxidation by promoting 1) inhibition of monocyte chemotaxis via monocyte chemotactic protein-1, 2) hydrolysis of lipid peroxide via paraoxonase enzyme, 3) reverse cholesterol transport via lecithin-cholesterol acyltransferase, and 4) direct inhibition of vascular endothelial activation via apolipoprotein A1.

The anti-atherogenicity of ginger extract could also be attributed to its direct anti-oxidative effects on macrophages as well as on plasma (LDL), (Rhode *et al*., 2007). Ginger extract was shown in another study to decrease macrophage oxidative responses. Supplementation of ginger extract to macrophages resulted in reduced capacity of the cells to oxidize LDL and reduced cellular uptake of Ox-LDL. It is indeed shown that ginger extract consumption reduces the cellular uptake of oxidized LDL, possibly due to steric modification of plasma lipoprotein receptors.

The LDL oxidation hypothesis of atherosclerosis development suggests that inhibition of LDL oxidation should result in the attenuation of the development of atherosclerotic lesions. It has been demonstrated that the reduced development of atherosclerotic lesions that consumed ginger extract was associated with reduced LDL oxidative state. This may be related to the fact that ginger extract can act as a free radical scavenger (Rhode *et al*., 2007; Coppola *et al*., 2007).

The extract significantly reduce LDL-cholesterol, LDL-cholesterol transports cholesterol to the arteries where they can be retained in arterial proteoglycans starting the formation of plaques, LDL-cholesterol possess a risk of cardiovascular disease when it invades endothelium and becomes oxidized since the oxidized form is more easily retained by the proteoglycans, thus increase of LDL-cholesterol is associated with atherosclerosis, heart-attack, stroke, peripheral vascular disease.
The importance of this cholesterol lowering effect is that the extract aids in the prevention or reduction of cardiovascular diseases and it is deduced that ginger rhizome extract significantly reduced serum lipids profile in Wistar albino rats when given at a dose of 200mg/kg for one month.

Data in **Table (4.5)** showed that CCl_4 intoxicated rats followed by ginger administration declared a small increment (14%) in body weight. It was noticed that the final body weight did not show the same trend of body weight gain compared to normal control. It is evident that negative control rats decreased their body weight by the amount that normal control rats gained. These results are in the same line with (Ahmed *et al*., 2009). Ginger extract helps the cells of the liver to regenerate and to perform their normal functions.

6. CONCLUSION

Ginger appears to be a herb that can be used for several purposes besides its use for its aroma in cooking, because it has nutrient and medical values.

Ginger and many of its chemical constituents have strong anti-oxidant actions, and it is shown that the crude ethanol extract of the plant has a hepatoprotective action on carbon tetrachloride-induced hepatoxicity and that is possibly due to its antioxidant effect and free radical scavenging activity.

Ginger constituents known to be beneficial against CCl_4 -mediated liver injury exert their protective action by toxin-mediated lipid peroxidation either via decreased production of carbon tetrachloride derived free radicals or through the antioxidant activity of the protective agents themselves.

Ginger has recorded the most potent effect in improving many biochemical parameters. As is found in this study, it down regulated free radical elevation, improved liver and cholestatic biomarkers, ameliorated hepatic marker enzymes, and reduced TC, TG, LDL-C but, increased HDL-C.

The hypocholesterolemic effect of ginger could have possibly resulted, at least in part, from the inhibition of cellular cholesterol biosynthesis observed after consumption of ginger extract.

Ginger is considered to be ‘‘**Generally Recognized as Safe’’** herbal medicinal plant.

The hepatoprotective effect of *Z. officinale* demonstrated in this study may explore its nutraceuticals role in human diet.

8. LIMITATIONS

The experiment was done under a number of constraints like:

- Lack of working experimental set up
- Lack of chemicals, kits, and other materials
- Scarcity of experimental animals(rats)

7. RECOMMENDATIONS

- Further studies involving animals and more studies involving human volunteers should be done before approving its use as a supplement for treatment of the liver diseases. Moreover precautions should be done before its trial on patients who have diseases that ginger may worsen, this is because natural substances can interact with medicine, be inappropriate for many health conditions and be harmful in high doses.
- More studies are also required on the kinetics of ginger and its constituents and on the effects of their consumption over a long period of time.
- As several metabolic diseases and age-related degenerative disorders are closely associated with oxidative processes in the body, the use of either ginger or one or more of its constituents as a source of anti-oxidants to combat oxidation warrants further attention.
- Investigating the histopathology of the liver organ is strictly recommended, because this gives an illustration about the architecture of liver cells.
- I could not assay the endogenous anti-oxidant enzymes due to the absence of the kits, but if these were available I think my research would be more relevant.
- The whole array of knowledge of medicinal plants is not something to be acquired only from formal and informal schooling. Individuals who have the interest in traditional medicinal plants as well as in their collection or in healing practices need to learn from professional practitioners. But these professionals are not willing to disclose their knowledge because they might be afraid of competition that may arise if the number of practitioners increases.
- Integration of traditional and modern medicine in Ethiopia, following either the Chinese, Japanese or Indian pattern or a combination of these, would help the promotion and development of local traditional medicine, which has been significantly contributing and is expected to contribute to the health care system and economic development of the country.
- Creating awareness on people who work in areas where CCl_4 is used as dry cleaning solvent and advising them to use ginger.

9. References

Abdulhamid Bedri Kello and Sebsib Belay **(2004).** Promoting production of medicinal plants for human and animal health in and around Bale Mountains National Park. Institute of Developments Research, Addis Ababa University.

Aeschbach, R., Loliger, J., Scott, B. C., Murcja, A **(2002)**. *Journal of Food and Drug Analysis.*3:128-130.

Aklilu Lemma **(1991)**. A preliminary report on the molluscicidal property of Endod (Phytolaca dodecandra). *Ethiopian Medical Journal.* 3: 187-190.

Albano.E,G. Bellorno,R.Carini,F.Biasi,G.Poli and M.U. Dianzan **(1987)**. Mechanisms responsible for carbon tetrachloride-induced perturbation of mitochondrial calcium homeostasis. *FEBS Lett.* 192:184-188.

Alnaqeeb, M.A.; Thomson, M.; Al-qattan, K.K.; Kamel, A.F.; Mustafa, T. and Ali, M **(2003)**. Biochemical and histopathological toxicity of an aqueous extract of ginger in female rats. *Kuwait J. Sci. Eng*. 30: 35-48.

Arneson Wendy and Brickel Jean **(2007)**. Clinical Chemistry: A laboratory perspective. 1:18-19.

Asai A, Miyazawa T **(2002)**. Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. *Life Sci*.67:2785–2793.

Aviram, M **(1996)**. Interaction of oxidized low density lipoprotein with macrophages in atherosclerosis, and the antiatherogenicity of antioxidants. Eur. J. Clin. Chem. Clin. Biochem. 34: 599–608.

Bhadauria M, Nirala SK, Shukla S **(2008)**. Multiple treatment of propolis extract ameliorates carbon tetrachloride induced liver injury in rats. *Food Chem Toxicol.*46: 2703-2712.

Bhandari, U., Sharma, J. N. & Zafar, R **(1998)**.The protective action of ethanolic ginger (*Zingiber officinale*) extract in cholesterol-fed rabbits. *J. Ethnopharmacol*. 61: 167–171.

Blazka ME, Wilmer JL, Holladay SD, Wilson R E, Luster MI **(1995)**. Role of Pro-inflammatory cytokines in acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol.*133:43-52.

Bode A, Ma W, Surh Y, Dong Z **(2001)**. Inhibition of epidermal growth factor-induced cell transformation and activator protein 1 activation by [6]-gingerol. *Cancer Res.* 61: 850.

Bourdi M, Masubuchi Y, Reilly TP **(2002)**. Protection against acetaminophen-induced liver injury and lethality by interleukin 10, role of inducible nitric oxide synthase. *Hepatology.* 35:289-298.

Bradford MM **(1976)**. A rapid and sensitive method for the quantitation of microgram

quantities of protein utilizing the principle of protein-dye binding*. Anal Biochem.*72: 248-254.

Brattin. W. J, S.D. Pencil, R. L. Waller, E.A. Glende and O.Recknagel **(1984)**. Assessment of the role of calcium ion in halocarbon hepatotoxicity. *Environ.Health.Perspect.*57:321-323. Brower V **(1998)**. Nutraceuticals, Nutritional Therapy, Phytonutrients, and Phytotherapy for Improvement of Human Health. *Nat Biotechnol.* 16:728–731.

Bruan, J.E.A. and D.L. Severson **(1992)**. Lipoprotein lipase released from myocytes is increased by decavandate but not insulin. *Am. J. Physiol*. 262:663-E670.

Buccolo, G. *et al.* **(1973)**. Quantitative determination of serum triglycerides by use of enzymes. Clin. Chem. 19:476-482.

Burk. R. f, Lane J. Mand Patel k **(1984)**. American society of clinical investigation.74:1996-2001.

Cerutti AA **(1991)**. Oxidant stress and carcinogenesis. *Eur J Clin Inves.*21: 1

Chalasani et al. **(2008)**. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology*.135:1924.

Chang CY, Schiano TD **(2007)**. Review article: drug hepatotoxicity. *Aliment Pharmacol Ther*.25:1135.

Coppola G, Novo S **(2007)**. Statins and peripheral arterial disease: effects on claudicating, disease progression, and prevention of cardiovascular events. Arch Med Res.38:479-88.

Cotran RS, KumarV, Collins T **(1999)**. Cellular pathology I: cell injury and cell death. In Robbins' Pathologic Basis of Disease, Cotran RS, Kumar V, Collins T (eds). 6thedn. WB Saunders: Philadelphia, PA. 75.

Davidsen, M. L.,W. urtz, S. Ø., Rømer, M. U., Sørensen, N. M. et al.**(2006)**. TIMP-1 gene deficiency increases tumour cell sensitivity to chemotherapy induced apoptosis. *Br. J. Cancer*. 95: 1114–1120.

Dawit Abebe and Estifanos Hagos **(1991)**. Plants as a primary source of drugs in the traditional health practices of Ethiopia. In PGRE, Engels, Jmm Hankes J.G. and Keleer Worede (eds.) Cambridge University.101-113.

Dawit Abebe **(2001)**. The role of medicine plants in Healthcare coverage of sustainable use of medicinal plants in Ethiopia. Proceedings of National Workshop on Biodiversity conservation and sustainable use of medicinal plants in Ethiopia.6-21.

Debela Hunde, Zemede Asfaw and Ensermu Kelbessa **(2004)**. Use and management of ethnoveterinary medicinla plants by indigenous people in Boosat, Welenchi area, Ethiop. J. *Biol. Sci*.3: 113-132.

Deiana M, Arouma OI, Bianchi M et al. **(1999)**. Inhibition of peroxinitite dependent DNA base modification and tyrocin nitration by the extra virgin olive oil derived antioxidant hydroxytyrosol. Free Radical Biol Med. 26: 762-769.

Desta Belachew **(1988)**. Ethiopian traditional herbal drugs potentiality and appropriate

utilization In the proceedings of the 8th International Conference of Ethiopian Studies.1: 763-765.

Diederichsen, A **(1996)**. Promoting the conversation and use of underutilized and neglected crops-3. *Coriandrum sativum*. Inter. Plant Resource Institute. 208-209.

Edwards, S **(2001)**. The ecology and conservation status of medicinal plants on Ethiopia. What do we know? 46-55.

Endashaw Bekele **(1978)**. Biochemical and morphological studies of the relationship of Eragrostis tef and some other Eragrostis species. M. Sc. Thesis, University of Birmingham, U. K.

Fassil Kibebew **(1998)**. The status and availability of data of oral and written knowledge on traditional health care in Ethiopia. A base line study. In: Conservation and sustainable use of medicinal plants in Ethiopia, IBCR.

Finkel T, Holbrook NJ **(2000)**. Oxidant, oxidative stress and biology of ageing. *Nature*.408: 239-247.

Flynn DL, et al. **(1986)**. Inhibition of human neutrophil 5-lipooxygenase activity by gingerdione, shogaol, capsaicin and related pungent compounds. *Prostaglandins Leukot Med.* 24:195-8.

Frankel, E **(1995)**. Nutritional benefits of flavonoids. International conference on food factors: Chemistry and Cancer Prevention, Hamamatsu, Japan. *Abstracts*.

Friedewald WT, Levy RI, Fredrickson DS **(1972)**. Estimation of the concentration of Low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem*. 18:499.

Fuhrman, B., Elis, A. & Aviram, M **(1997)**. Hypocholesterolemic effect of lycopene and b-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages. *Biochem. Biophys. Res.* Commun.233: 658–662.

Galila AY, Nihal ME Eman FE **(2007)**. Amelioratic Effect of Zingiber Officinale on Experimentally Induced Liver Fibrosis in Rats. J Med Res Inst.28: 154-5

Govindarajan V **(1982)**. Ginger-chemistry, technology and quality evaluation: part 1. *Crit Rev Food Sci Nutr.* 17:1-96.

Gowri Shankar GNL, Manavalan R, Venkappayya D, Raj CD **(2008)**. Hepatoprotective andantioxidant effects of *Commiphora berryi* (Arn) Engl bark extract against CCl_4-induced oxidative damage in rats**.** *Food Chem Toxicol.* 46: 3182–3185.

Habib SH, Makpol S, Abdul Hamid NA, Das S, Ngah WZ, Yusof YA **(2008)**. Ginger extract (*Zingiber officinale*) has anti-cancer and anti-inflammatory effects on ethionineinduced hepatoma rats. *Clinics (Sao Paulo).* 63: 807-813.

Hailemichael G, Tesfaye K **(2008)**. The effects of seed rhizome size on the growth, yield and economic return of ginger (*Zingiber officinale* Rosc.). *Asian J. Plant Sci*. 7: 213-217.

Hamed MA **(2011)**. Metabolic profile of rats after one hour of intoxication with a single oral

dose of ethanol. *J Pharmacol Toxicol.* 6: 158-165.

Deshmukh V.N.*et al*. **(2011)**. Int. J. *Pharm Tech Res*. 3: 298-308.

Halliwell B, Chirico S **(1993)**. Lipid peroxidation: its mechanism, measurement and significance. *Am J Clin Nut.* 57: 715–725.

Halliwell B, Gutteridge JMC **(1998)**. Free radical in biology and medicine, 3rd Edition. Oxford University Press, London Chapter 3.

Handa SS **(1991)**. Plants as drugs. *The Eastern Pharmacist.* 34:79-85.

Hareya Fassil **(2005)**. Beyond plants, professionals and parchments. The role of home-based medicinal plant use and traditional health knowledge in primary healthcare in Ethiopia. Ethnobotany Research and Applications. 3: 37-49.

Hyogo H, Yamagishi **(2008)**. Advanced glycation end products (AGEs) and their involvement in liver disease. *Curr Pharm Des.*14: 969-972.

Ivanova D, Gerova D, Chervenkov T et al. **(2005)**.Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J Ethnopharmacol.*95: 145-150.

Jadon A, Bhadauria M, Shukla S **(2007)**.Protective effect of *Terminalia belerica* Roxb. And gallic acid against carbon tetrachloride induced damage in albino rats. *J Ethnopharmacol.*109: 214-8.

Jandrasssic Gruff **(1981)**. Analysis for total and direct bilirubins in serum with a centrifugal analyzer.27:1410-1416.

Jeong HG, You HJ, Park SJ, Moon AR, Chung YC, Kang SK, Chun HK **(2002)**. Hepatoprotective effects of 18- glycyrrhetinic acid on carbon tetra chloride induced liver injury inhibition of cytochrome P450 2E1 expression. *Pharmacol Res.* 3:46.

Junnila M, Barak AJ, Rahko T **(1998)**. Betaine reduces hepatic lipidosis induced by carbon tetrachloride in Sprague-Dawley rats. *Vet Hum Toxicol*. 40: 263–266.

Kaur C, Kapoor HC **(2002)**. Antioxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Technol*. 37: 153-161.

Kikuzaki H, Nakatani N **(1993)**. Antioxidant effects of some ginger constituents. *J Food Sci.*58: 1407-1410.

Kikuzaki H, Usuguchi J, Nakatani N **(1991)**. Constituents of Zingiberaceae I. Diarylheptanoid from the rhizomes of ginger (Zingiber officinale Roscoe). *Chem Phar Bull.*39: 120.

Kiuchi F, et al. **(1992)**. Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids. *Chem Pharm Bull* (Tokyo).40:387-91.

Kumaravelu P, Dakshinamoorthy DP, Subramaniam S, Devarajt H, Devaraj NS **(1995)**. Effect of eugenol on drug-metabolizing enzymes of carbon tetrachloride-intoxicated rat liver. *Biochem Pharmacol.*49: 1703-1707.

Lambert, John D.H., Ryden, Per A. and Esikuri, Enos E **(2006)**.Capitalizing on the Bio-economic value of multi-purpose medicinal plants for the rehabilitation of dry lands in Sub-Saharan Africa. Global Environment Facility.

Landbo AK, Meyer AS **(2001)**. Ascorbic acid improves the antioxidant activity of European grape juices to inhibit lipid peroxidation of human LDL in vitro. *Int J Food Sci.*36: 727-736. Lee Goldman and Schafer Andrew Rev. ed. of Cecil medicine 24th ed. Columbia University, 2008:1349-1351.

Lee SE, Hwang HJ, Ha JS et al. **(2003)**. screening of medicinal plant extracts for antioxidant activity. *Life Sci.*73: 167-179.

Lee WM **(2003)**. Drug-induced hepatotoxicity. *N Engl J Med.*349:474.

Leonard TB, Neptun DA, Popp JA **(1984)**. Serum gamma glutamyl transferase as a specific indicator of bile duct lesions in the rat liver. *Am J Pathol.* 116: 262-269.

Lines JG, Raines DN **(1970)**. Refractometric determination of serum concentration: 2. Comparision with biuret and kjeldahl determination. *Ann Cli Biochem.*7:6.

Lopes-Virella, M. F., P. Stone, S. Ellis and A. J. Coldwell **(1997)**.Cholesterol determinations in high density lipoproteins separated by three methods. *Clin. Chem*.23:882-884.

Lynch T, Price A **(2007)**.The effect of cytochrome P- 450 metabolism on drug response, interactions and adverse effects. *Amer Fam Physi.* 76:391-396.

Mac Cay P, Lai E, Poyer J **(1984)**. Oxygen-and carboncentered free radical formation during carbon tetrachloride metabolism observation of lipid radical *in-vivo* and *in-vitro*. *J Biol Chem.* 259:2135.

Manna P, Sinha M, Sil PC **(2006)**. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Comp Alt Med.*6:1472-6882.

Mansour HH, Hafez HF, Fahmy, NM **(2006)**.Silymarin modulates cisplatin-induced oxidative stress and hepatotoxicity in rats. *J Biochem Mol Biol.*39: 656-661.

Maor, I., Hayek, T., Coleman, R. & Aviram, M **(1997)**.Plasma LDL oxidation leads to its aggregation in the atherosclerotic apolipoprotein E-deficient mice. Arterioscler. Thromb. Vasc. *Biol*.17: 2995–3005.

Mascolo N, Jain R, Jain S **(1989)**. Ethenopharmacological investigation of ginger (Zingiber officinale). *J. Ethenopharmacology.*129-140.

Matsuda A, Wang Z, Takahashi S, Tokuda T, Miura N, Hasegawa J **(2009)**. Up regulation of mRNA of retinoid binding protein and fatty acid binding protein by cholesterol enriched-diet and effect of ginger on lipid metabolism. *Life Sci.* 84: 903-907.

Meshkibaf MH, Ebrahimi A, Ghodsi R, Ahmadi A **(2006)**. Choronic effects of lamotrigine on liver function in adult male rats. *Ind J Clin Biochem* 2006; 21: 161-64.

Meyer SA, Kulkarni AP **(2001)**. In: Introduction to biochemical toxicology. 3rd Ed., John Wiley and Sons, New-York.

Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S **(1998)**. Effect of HD-03, a herbal formulation, on the antioxidant defense system in rats. *Phytother Res.*12: 114–117.

Moron MS, Depierre JW, Mannervik B **(1979)**. Level of glutathione, glutathione reductase and glutathone-S-transferase activities in rat lung and liver. *Biochimica Biophysica* Acta.582: 67-78.

Mujumddar AK; Upadhye AS; Pradhan AM **(1998)**. *Ind. J. Pharm. Sci*. 60: 363.

Murugaiah, J. S., N. Nalini, V. P. Menon and N. Namasivayam **(1999)**. Effect of ginger (*Zingiber officinale R.*) on lipids in rats fed atherogenic diet. *J. Clin. Bio.Nutr*. 27:79-87.

Mustafa J, Srivastava K, Jensen K **(1993)**. Drug development report: 9. Pharmacology of ginger, Zingiber officinale. *J. Drug Development.*25-39.

Mutter D, Soler L, Marescaux J **(2010)**. Recent advances in liver imaging. *Expert Rev Gastroenterol Hepatol*.4:613-621.

Naito, H K **(1984)**. High-density lipoprotein (HDL) cholesterol. Clin. Chem. The C.V. Mosby Co. St.Louis.Princeton. 437:1207-1213.

Nakazawa T, Ohsawa K **(2002)**. Metabolism of [6]-gingerol in rats. *Life Sci*.70:2165–2175.

Naora K, Ding G, Hayashibara M, Katagiri Y, Kano Y, Iwamoto K **(1992)**. Pharmacokinetics of [6]-gingerol after intravenous administration in rats with acute renal or hepatic failure. *Chem Pharm Bul*l (Tokyo).40:1295–1298.

Narasimhanaidu K. and Ponnaian S. M. P **(2006)**. Antihyperglycaemic and Antioxidant Effect of gingerol, Polyphenolic flavonoids in *streptozotocin* - induced Diabetic Wistar rats. J. Basic and Clin. *Pharmacol.and Toxicol*. 98: 97-103.

Ness, G. C., Zhao, Z. & Lopez, D **(1996)**.Inhibitor of cholesterol biosynthesis increase hepatic low density lipoprotein receptor protein degradation. *Arch. Biochem Biophys*.325: 242–248.

Nishikimi M, Rae NA, Yagi K **(1972)**.The occurrence of superoxide anion in the action of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophys Res Commun.*46: 849-853.

Opoku AR, Ndlovu IM, Terblanche SE, Hutchings AH **(2007)**.*In vivo* hepatoprotective effects of *Rhoicissus tridentata* subsp. cuneifolia, a traditional Zulu medicinal plant, against CCl_4-induced acute liver injury in rats. *South African J Botany.*73: 372–377.

Pan, M. H., Hsieh, M. C., Hsu, P. C., Ho, S. Y. et al **(2008)**. 6-Shogaol suppressed lipopolysaccharide-induced upexpression of iNOS and COX-2 in murine macrophages. *Mol. Nutr. Food Res*.52: 1467–1477.

Paraskevas K., et al **(2011)**. Comparison of fibrate, ezetimibe, low-and high-dose statin therapy for the dyslipidemia of the metabolic syndrome in a mouse model. *Angiology*. 144-154.

Park KK, Chun KS, Lee SS, Surh YJ **(1998)**. Inhibitory effect of [6]-gingerol, a major pungent principle of ginger, on phorbol ester-induced inflammation, epidermal ornithine decarboxylase activity and skin tumor promotion in ICR mice. *Cancer Lett.*129:139-44.

Parola M, Lonarduzzi G, Biasi F, et al **(1992)**. Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis. *Hepatology*. 6:1014–1021.

Poli, G, Albano E, and Dianzani M.U **(1987)**.The role of lipid peroxidation in liver damage. *Chem. Phys. Lipids*. 45:117-142.

Pratap A., et al **(2010)**. "Inhibition of endogenous hedgehog signaling protects against acute liver injury after ischemia reperfusion". *Pharm Res*. 27:2492-2504.

Prior RL **(2003)**. Fruit and vegetables in the prevention of cellular oxidative damage. *Am J Clin Nutr*. 78: 570-578.

Rao SB; Mehendale HM **(1989)**. *Biochem. J.*262: 721-725.

Recknagel RO, Glende EA, Dolak JA, Walter RL **(1989)**. Mechanisms of carbon tetrachloride toxicity. *Pharmacol Ther.*43: 139–154.

Recknagel RO, Glende EA **(1973)**. Carbon Tetrachloride hepatotoxicity: an example of lethal damage. *CRC Crit Rev Toxicol* 1973; 2: 263–297.

Reyes-Gordillo K, Segovia J, Shibayama M, Vergara P, Moreno MG, Muriel P **(2007)**. Curcumin protects against acute liver damage in the rat by inhibiting NF-kappa B, proinflammatory cytokines production and oxidative stress. *Biochem Biophys Acta.*1770: 989-996.

Rhode, J., Fogoros, S., Zick, S., Wahl, H. et al **(2007)**. Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. *BMC Complement Altern*. Med. 7:44–52.

Rice ME, Shelton E **(1957)**. Comparison of the reduction of two tetrazolium salts with succinoxidase activity of tissue homogenates. *J Nat Cancer Inst.*18: 117-125.

Romero FJ, Bosch-Morell F, Romero MJ, Jareño EJ, Romero B, Marín N, Romá J**(1998)**. Lipid peroxidation products and antioxidants in human disease. *Environ Health Perspectives.*106: 1229–1234.

Roschlau, P.*et al* **(1974)**. *Clin. Chem. Clin Biochem*.12:403.

Rubinstein D, Roska AK, Lipsky PE **(1986)**. Liver sinusoidal lining cells express class II major histocompatibility antigens but are poor stimulators of fresh allogeneic T- lymphocytes. *J. Immunol*. 137:1803-1810.

Schmidt LE et al **(2002)**. Acute versus chronic alcohol consumption in acetaminophen-induced hepatotoxicity. *Hepatology*.35:876.

Shanmugasundaram P, Venkataraman S **(2006)**. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) *Heine acanthaceae* root extract. *J Ethnopharmacol.*104: 124–128.

Sharma N, Shukla S **(2010)**. Hepatoprotective potential of aqueous extract of *Butea monosperma* against CCl_4 induced damage in rats. *Exp.Toxicol Pathol.*

Sherlock S, Dooley J **(2002)**. Hepatic cirrhosis, in Diseases of the Liver and Biliary System, Chapter 21. Malden, MA: *Blackwell Science*.365–380.

Sheweita SA, Abd El-Gabar M, Bastawy M **(2001)**. Carbon tetrachloride changes the activity of cytochrome P450 system in the liver of male rats: role of antioxidants. *Toxicology*.169: 83–92.

Shukla Y, Singh M **(2007)**. Cancer preventive properties of ginger: A brief review. *Food Chem Toxicol*.45:683-90.

Srinivasan, K. & Sambaiah, K **(1991)**. The effect of spices on cholesterol 7-alpha-hydroxylase activity and on serum and hepatic cholesterol levels in the rat. *Int. J. Vitam. Nutr. Res*.61: 363–369.

Trease GEM, Evans WC **(1989)**. In: Trease and Evans *pharmacognosy*, 13th ed, Bailliere Tindall, London.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J **(2007)**. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.*39: 44-84.

Wang MY, Anderson G, Nowicki D, Jensen J **(2008)**. Hepatic Protection by Noni Fruit juice against CCl_4 induced chronic liver damage in female SD rats. *Plant Foods Hum Nutr.*63: 141–145.

Wang SY, Jiao H **(2000)**. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. *J Agr Food Chem.*48: 5672-5676.

Wei Y, Jun L, Qiang C **(2004)**. Effect of losartan, an angiotension II antagonist, on hepatic fibrosis induced by CCl_4 in rats. *Dig. Dis. Sci*.49:1589.

Wolf PL **(1999)**. *Ind. J. Clin. Biochem*.14, 59-64.

Yadav NP, Pal A, Shanker K, Bawankule DU, Gupta AK, Darokar MP, Khanuja SPS **(2008)**. Synergistic effect of silymarin and standardized extract of *Phyllanthus amarus* against CCl_4-induced hepatotoxicityin *Rattus norvegicus*. *Phytomedicine.*15: 1053–1061.

Zuwała-Jagiełło J, Pazgan-Simon M, Krzysztof Simon K, Maria Warwas M **(2011)**. Advanced oxidation protein products and inflammatory markers in liver cirrhosis: a comparison between alcohol-related and HCV-related cirrhosis. *Acta Biochimica Polonica.*58: 59-65.