CHAPTER-1.
INTRODUCTION

Over the last three to four decades, use of alternative system of medicines based on Ayurveda, Siddha, Unani, etc. in India [Kapoor, 1990] and similar traditional system in other countries has been growing in a rapid pace. Herbal medication is described as a branch of science wherein plant based formulations is used to relieve illnesses. It is also referred to as botanical medicine or phytomedicine. These days phytotherapy has been brought as more accurate synonym of natural or botanical medication. In the early 20th century herbal medicinal drug was top healthcare device as antibiotics or analgesics had been not as yet discovered. With the arrival of allopathic system of medicine, natural medicine gradually misplaced its popularity among people, that's based on the fast therapeutic effect of synthetic medicine [Singh, 2007].

Recently there was a shift in universal trend from synthetic to natural remedy, which can be stated “Go back to Nature”. Medicinal plants have been recognised for millennia and are highly esteemed all around the globe as a wealthy source of healing agents for the prevention of diseases and ailments [Sharma et al., 2008].

1.1. Phytochemistry and medicinal plants

1.1.1. Traditional Medicine and Drug discovery

Thousands of years human have relied on natural product as the primary source of medicines. Plants form the basis for traditional medicine system. These plants based traditional medicine system continue to play an essential role in health care and it has been estimated by WHO that approximately 80% of the worlds inhabitant rely mainly on traditional medicine for their primary health care.
Plant products also play an important role in the health care system of remaining 20% of population, mainly residing in developed countries. Analysis of data on prescriptions dispensed from community pharmacist in the United State from 1959-1980 indicates that about 25% contained plant extracts or active principle derived from higher plants and at least 119 chemical substances, derived from 90 plant species can be considered as important drugs currently in use [Farnsworth et al., 1985]. Of these 119 drugs, 74% were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine.

The isolation of the antimalarial drug quinine from the bark of Cinchona species (ex. C.offinalis) were reported in 1820 by the French pharmacist, Caventon and Pelletier. The bark had long been used by indigenous group in the Amazon region for the treatment of fever and it was first introduced in Europe in early 1600’s for the treatment of malaria. Quinine formed the basis for synthesis of the commonly used antimalarial drugs such as chloroquinine and mefloquinine. The analgesic, morphine, isolated in 1816 by the German pharmacist, Serturner from the opium poppy (Papaver somniferum) [Buss and Waugh, 1995].

Other significant drugs developed from traditional medicinal plant include: the antihypertensive agent, reserpine, isolated from Rauwolfia serpentina used in Ayurvedic medicine for the treatment of snake bite and other ailments (Kpoor, 1990), ephedrine from Ephedra sinica, a plant long used in traditional medicine was introduced in Western medicine in 1923 and is the basis for the synthesis of anti-asthma agents salbutamol and salmetrol [Arvigo, 1993]
1.1.2. Approved Natural Product-Based Drugs

The analysis of drugs approved by the FDA in USA in 10 years period (1983-1992) conducted by Cragg and associate found that 157 of 520 drugs (30%) approved were natural products or their derivative [Cragg et al., 1997]. When focused efforts are made to discover natural product for clinical use the success level rises dramatically, thus in the same period 61% anticancer agents approved were natural product and their derivatives. The Western European phytochemical market in prescription was $2.2 billion (70% in Germany) in 1989 and are growing figure shows that the trade in plants used in Europe for non-conventional medication is increasing by 15%-20% every year, with an import value of $ 3.6 billion in 1995 [Ho and Kim, 2002]. However the exact value of medicinal phytochemical drug market is difficult to establish because although some of these products sold via prescription, others sold over the counter by a range of different retailers. Among the 25 best-selling drugs in the world 30% comes from natural product. In 1996, six of the top twenty pharmaceutical drugs sold were natural products and more than 50% of these linked directly to the natural products [Kong Jim Goh et al., 2003].

A total of approximately 38 natural product based drugs were approved and launched in the market during the period 2000 to 2010. This section deals with these authorised drugs as per categorized diseases areas along with infectious disease area (15 drugs), oncology (7 drugs), neurological disease area (7 drugs), cardiovascular and metabolic disease area (4 drugs), diabetes (1 drug), and some other diseases areas (4 drugs). In addition, all these approved drugs are summarized. [Goutam, 2010]
1.1.3 Phytochemicals and their Medicinal Uses

Medicinal plants are of awesome importance to the health of people and communities. The medicinal value of these plants is hidden in some chemical substances that produce a definite physiological action on the human body, and these chemical substances are known as secondary metabolites which can also be called phytochemicals [Hill, 1974]. The plant secondary metabolites vary from plant to plant, thus the plant kingdom provides a tremendous reservoir of various chemical substances with potential therapeutic purposes. These secondary metabolites constitute important sources of pharmaceutical drugs [Adebanjo and Adewumi, 1983]. The following are some of the active constituents occurring naturally in medicinal plants:

Alkaloids

Alkaloids are heterogeneous group of compounds which contain one or more nitrogen atom in acyclic system. They are basic compounds that are derived from plants. In nature, alkaloids exist in large proportions in the seeds and roots of plants and often in combination with vegetable acids [Madziga et al., 2010]. The investigation of bioactivity of alkaloids reveals that they are used medically as antispasmodic, mydriatic, local anesthetic drug [Harborne, 1973]. They are mostly used as anti-depressant (morphine), stimulants (caffeine), anesthetics (cocaine), anti-tumor (vinblastine), anti-malaria (quinine), anti-bacteria (berberine) and amoebicide (emetine) [Heinrichn et al., 2004]. Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against diseases and endurance against stress. They also have a protective role in animals [Edeoga et al., 2006].
Saponins

There are two types of sapogenin and these include: steroid saponins and triterpene saponins. Saponins are extremely poisonous, as they cause hemolysis of blood and are known to cause cattle poisoning [Kar, 2007]. These are glycosides of both triterpenes and sterols. They are the surface active agents and they possess soap-like properties [Trease and Evans, 1989]. It has been discovered that saponins and other flavonoid compounds at low concentrations inhibited the growth micro-organisms and they can also act as bactericidal agents at higher concentrations. [Sofowora, 1993].

Saponin is used in the manufacture of shampoos, insecticides and various drug preparations and in synthesis of steroid hormones [Okwu, 2004]. Generally, saponins are toxic, but researches have recently shown that consumption of saponins by human beings may be beneficial in reducing heart disease. The presence of steroidal saponins could develop resistance to viral diseases. Finar (1989) reported that, saponins had expectorant action which is very useful in the management of upper respiratory tract inflammation. So these plants may be used to treat various ailments.

Terpenoids

Terpenoids includes hydrocarbons of plant origin of general formula (C5H8)n and are classified as mono-, di-, tri- and sesquiterpenoids depending on the number of carbon atoms. Monoterpenes include terpinen-4-ol, thujone, camphol, eugenol and menthol. Diterpenes are classically considered to be resins. The triterpenes include steroids, sterols and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Sesquiterpenes like monoterpenes, are major components of many essential oils [Martinez et al., 2008].
**Glycosides**

Glycosides in general, are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxyl (occasional thiol) compounds (invariably monohydrate in character). Chemically, glycosides contain a carbohydrate (glucose) and a non-carbohydrate part (aglycone or genins). They are complex groups which can be broken down to yield one or more sugar (glycones) [Kar, 2007; Firn, 2010].

**Steroids**

Plant steroids (or steroid glycosides) also referred to as “cardiac glycosides” are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs [Firn, 2010]. Therapeutically, steroids contribute cardiotonics, vitamin D precursors, anti-inflammatory agents (corticosteroid) and anabolic agents (androgen) [Trease and Evans, 1992].

**Tannins**

They are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannins are found in the root, stem, bark and outer layers of plant tissue. They are acidic in reaction, and the acidic reaction is attributed to the presence of phenolics or carboxylic group. They form complexes with proteins, carbohydrates, gelatin and alkaloids Tannins are used as anti-septic and as anti-microbial agents in plants. Two basic groups are usually recognized: hydrolysable tannins and condensed tannins. Hydrolysable tannins, upon hydrolysis, produce gallic acid and ellagic acid and these are called gallotannins or egallitannins. [Kar, 2007].
Phenols

These are also called poly phenols or phenolic compounds and they are widely found throughout the plant kingdom. They are chemical compounds that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections [Pwupponeu-Pima et al., 2008]. They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavones, flavonones, xanthones and catechins) and (iii) non- flavoned polyphenolics. [Kar, 2007]. They have an aromatic ring bearing one or more hydroxyl groups. The most important role may be in plant defence against pathogens and herbivore predators. They are beneficial to man as powerful anti-oxidants, stress modifiers, anti-allergic agents, anti-viral compounds, stimulant of protein synthesis, anti-inflammatory agents, vaso-propertive activity, diuretic, anti-spasmodic, anti-bacterial and anti-fungal [Trease and Evans, 1992]. Phenols are reported anti-tumour agents and are known to exhibit anti-viral and antimicrobial activities, hypotensive effects and anti-oxidant properties [Arts and Hollman, 2005].

Flavonoids

Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally they are made of more than one benzene ring (a range of C15 aromatic compounds) and numerous reports support their use as anti-oxidants or free radical scavengers [Kar, 2007]. The compounds are derived from parent compounds known as flavans. Over four thousand flavonoids are known to
exist as pigments in higher plants. More recent research has enabled scientists to group them into classes on the basis of similar protective functions as well as individual physical and chemical characteristics of the molecules. Flavonoids have been reported to be synthesized by plants in response to microbial infection and have been shown to have anti-bacterial activities. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity [Aiyelagbe and Osamudiamen, 2009].

1.2 Acute toxicity study

In the assessment and evaluation of the toxic characteristic of a substance determination of acute oral toxicity is usually an initial step. Pharmacologist and physicians were very much concerned with potency of the drugs and they began to carry out acute toxicity test in laboratory animals more than hundred years ago. Median lethal dose concept was introduced by Trevan (1927) for standardizing insulin, diptheria toxin and extract of Digitalis. Since then to provide some information on the acute toxicity of a chemical substance it has been a common practice to find out LD$_{50}$ values in experimental animals [Marshal, 1981]. The LD$_{50}$ has been defined as a numerical index, which gives some information about acute toxicity of chemical substances in experimental animals. However a lot of criticisms have been done on the validity of LD$_{50}$ test. These test do not indicate the cause of death, which sometime results merely from the sheer bulk of the dose [Rowan, 1981]. In many cases LD$_{50}$ results is unnecessary waste of experimental animal [Brown, 1983].

Brown (1988) said “Too many people have abused the validity of LD$_{50}$ values by failing to apply good scientific judgment in their usage”. There is a common misconception that LD50 test is synonymous with acute toxicity testing.
LD$_{50}$ is a numerical index, whereas acute toxicity is concerned with wider implication of the response to short term and exposure to products than just a numeric number. An acute toxicity experiment is more informative and useful than an experiment designed solely to determine the LD$_{50}$ value.

1.3. The Liver

The liver is the largest internal organ in the body with four lobes of different size and shape and surrounded by a firm layer of connective tissue called Glisson’s capsule encloses the whole liver. It gets the nutrients and oxygen through the main hepatic blood vessels, portal vein, and hepatic artery. The lobes of the liver are comprised of numerous functional devices referred to as lobules. Lobules are the useful units of the liver, each lobule is made up of either parenchymal cells (hepatocytes), which are the basic metabolic cells or nonparenchymal cells [hepatic stellate cells (HSC), kupffer cells and sinusoidal endothelia cells] [Gressner, 1991]

![Figure 1: Structure of the liver A) (Source: anterior aspect, (B) inferior aspects Adapted from Saladin (2003).](image)

Liver is multifunctional largest organ, responsible for many vital life functions. It plays a great role in carbohydrate, protein and fat metabolism, synthesis of bile components, detoxification of blood and storage of vitamins and minerals. It also performs many activities that are critical for survival such as synthesis of blood clotting factors, creation of proteins necessary for growth and metabolic processing of
most drugs and toxins. It likewise has an amazing part in the support, execution and
directing homeostasis of the body. It is included with all the biochemical pathways to
development, battle against illness, supplement supply, energy provision and
reproduction [Ahsan et al., 2009].

1.3.1. The major functions of liver;

1.3.1.1. Storage:

The liver provides storage of many essential nutrients, vitamins, and
minerals obtained from blood passing through the hepatic portal system. For instance,
the liver stores glucose as glycogen, and converts it again to glucose when required.
The liver can convert excess glucose and amino acids into fatty acids for storage. If
the grant of glycogen is drained, the liver can additionally synthesize glucose from
amino acids, lactate, and glycerol [Allen, 2002]. Other nutrients stored in the liver
include iron and vitamin A [Nelson and Cox, 2005].

1.3.1.2. Synthesis:

The liver synthesizes numerous proteins that circulate inside the blood.
These consist of albumin, coagulation elements, alpha1-antitrypsin, very low density
lipoprotein, insulin-like development variables, thrombopoietin, and so on. It also
produces glucose from certain amino acids, lactate or glycerol (gluconeogenesis),
glycogen from glucose (glycogenesis) and glucose from glycogen (glucogenolysis).
The liver synthesizes cholesterol and produces bile which acts as a cleanser, and
separates fats into smaller segments, so they can be digested in the small intestine.
Bile also gives a route to the liver to eliminate wastes, consisting of bilirubin,
cholesterol, and toxins. Finally, the liver can synthesize non-essential amino acids
when needed by the body [Allen, 2002].
1.3.1.3 Biotransformation and elimination:

The liver is central to the metabolism of virtually every foreign substance. Most medications and xenobiotics are lipophilic, empowering them to pass the membranes of intestinal cells [Weinshilboum, 2003]. Poisons are detoxified by the liver’s capacity to metabolize lipophilic compounds [Nelson and Cox, 2005]. Medications are rendered more hydrophilic by biochemical procedures in the hepatocyte, yielding water-soluble product that are discharged in urine or bile [Weinshilboum, 2003]. This hepatic biotransformation includes oxidative pathways, mainly by means of way of the cytochrome P-450 enzyme device [Guengerich, 2001]. However, the reaction to which some xenobiotics are subjected to in the liver actually increases their toxicity [Granner et al., 2003]. The liver also processes excess amino groups into urea and other products to be excreted by the kidney.

Thus, the liver serves as the body’s distribution center, exporting nutrients in the correct proportion to other organs, smoothing out fluctuations in metabolism caused by intermittent food intake and processing products for elimination [Nelson and Cox, 2005]. Failure of these metabolic functions represents the basic pathophysiology of all forms of liver disease [Allen, 2002].

The liver has protective mechanisms against disease. It can regenerate itself by repairing or replacing injured tissue. Furthermore, it has many cell units responsible for the same task. If one area is injured, other cells will perform the functions of the injured section indefinitely or until the damage has been repaired. However, these mechanisms may be overpowered [C-Health fact sheet, 2013].

1.3.2. Liver disease

Liver ailment is one of the principal reasons of morbidity and mortality in public, affecting people of all ages. About 20,000 deaths appear each and every
year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year (Gupta and Misra, 2006). According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually [Negi et al., 2008].

Depending on the duration of the disease the liver diseases are classified as acute or chronic. If the disease does not exceed six months it is considered as acute liver disorder while diseases of longer duration are classified as chronic. Acute viral hepatitis and drug reactions account for the majority of cases of acute liver disease. Hepatitis A and B are the commonest causes of viral hepatitis in Europe and hepatitis E is common in India. Hepatitis C is not usually recognised as an acute infection because it rarely causes jaundice at this stage. Chronic liver damage is a worldwide common pathology characterized by inflammation and fibrosis that can lead to chronic hepatitis, cirrhosis and cancer [Tessitore and Bollito, 2006; Kohle et al., 2008].

Chronic hepatitis or long term intoxication can severely injure hepatic cells. Initially, the damaged cells are denatured, but subsequently transformed to hypertrophic fibrosis and necrosis, and eventually may progress to hepatoma. Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, schistosomiasis, etc; or chemical factors such as CCl4 administration, alcohol intake, etc. were known to contribute to
liver fibrosis. Hepatic fibrosis is major features of a wide range of chronic liver injuries including metabolic, viral, cholesteric and genetic disease.

The failure of bile salt excretion in cholestasis leads to retention of hydrophobic bile salts within the hepatocytes and causes apoptosis and/or necrosis [Miyoshi et al., 1999].

Oxidative stress has been implicated in the pathogenesis of various liver diseases including alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis C [Seki et al., 2005; Kitase et al., 2005]. In many patients, hepatitis such as non-alcoholic fatty liver disease becomes chronic and eventually progresses to more serious liver pathologies, such as fibrosis, cirrhosis, or even carcinogenesis, causing devastating economic losses and mortality [Albano et al., 2005]. Drug/chemical-mediated hepatic injury is the common sign of drug toxicity [Lee, 2003] and accounts for greater than 50% of acute liver failure cases. Hepatic damage is the largest obstacle to the development of drugs and is the major reason for withdrawal of drugs from the market [Cullen and Miller, 2006]. Drug-induced liver disease can be predictable (high incidence and dose-related) or unpredictable (low incidence and may or may not be dose-related). Unpredictable reactions, also referred to as idiosyncratic, can be viewed as either immune-mediated hypersensitivity or nonimmune reactions. Most potent predictable hepatotoxins are recognized in the animal testing or clinical phase of drug development.

The liver is the most important site of drug metabolism. However, many drugs are known to cause hepatic injury. Conventional and synthetic drugs used within the remedy of liver diseases are from time to time inadequate and might have serious adverse effects. Steroids, vaccines, and antiviral drugs, had been used as treatment options for liver pathologies, have potential damaging side-effects,
specifically if administered chronically or sub-chronically. Current medical remedies for those liver diseases are regularly useless, and consequently efforts are being made to find new effective medications [Seeff et al., 2001]. Developing pharmacologically effective retailers from natural merchandise has end up a new fashion with the aid of distinctive feature of their little toxicity or few side consequences. There are few plant derived drugs in the market which are used for the liver disorders.

**Silymarin**

Silymarin, derived from the seeds of Silybum marianum L., commonly known as milk thistle, has been used for centuries as a natural remedy for liver and biliary tract diseases [Saller et al., 2001]. Milk thistle protects and regenerates the liver in most liver diseases such as cirrhosis, jaundice, and hepatitis [Flora et al., 1998]. Silymarin provides true protection in a number of models of experimental liver disease.

**Limitations**

Silymarin is insoluble in water and typically administered as a sugar coated tablet [Thakur, 2002] or as an encapsulated standardized extract. Side Effects Silymarin has low level of toxicity. Although, silymarin has a good safety record, there are few reports of gastrointestinal disturbances and allergic skin rashes [Negi et al., 2008]

**Liv-52**

Liv-52 was introduced in 1954 as a specially formulated Ayurvedic herbal remedy for the treatment of viral hepatitis and has been widely prescribed for infective hepatitis since then [Mukerjee and Dasgupta, 1970]). Experimentally, Liv-52 avoided injurious results of carbon tetrachloride and different poisonous materials on the liver. Liv.52 is available as tablets and syrup containing the following herbs:
Capparis spinosa; Cichorium intybus; Solanum nigrum; Terminalia arjuna; Cassia occidentalis, Achillea millefolium; Tamarix galica and Phyllanthus amarus. These herbs are processed and formulated in accordance to the principles of Ayurveda, which are aimed toward improving efficacy and averting toxicity [Charak and vimanasthan, 1981].

1.3.3. Hepatotoxicity Inducing Agent

Many xenobiotics like chemicals, Drugs, house hold things, herbs and environmental chemicals have been known to induce hepatotoxicity. Some list of chemicals is follow that are responsible for hepatotoxicity [Zimmerman 1978; Kuntz 2008; Sturgil et al., 1997].

- Industrial chemical: CCl4, Trtra chloroethane Di phenyleoxide Chloroform, Ethylene dichloride, Arsenic, Antimony, Copper, Hydrezines
- House hold thing: Antifreeze Dry cleaning fluids Glue, Stamping Ink Paint Products,Polishes, Paint remover, Wax
- Pesticides: Organochloride, insecticide Herbicide, fungicide Thallium, warfarin, Copper salt , DDT
- Pollutant chemical in food and water: Polychloridated Biphenyls Polybrominated biphenyls Chloroalkane
- Plant Extract: Pyrrolizidine alkaloids, Pennyroyal, Kava Kava, Broom corn, Bajiaolian,Margosa Oil, Jin Bu Huan, Chaparral
- Drugs: Paracetamol (Acetaminophen-APAP), Acetophenazine Maleate Amrinone Lactate, Azacitidine, Asparaginase, Blenoxane
- Anti-Tuberculosis drug: Isoniazid, Rifampicin, Rifabutin Pyrazinamide Ethionamide, Prothionamide, Para-aminosalicylic acids
1.3.4. Mechanism of CCl₄-Induced Hepatotoxicity

The hepatotoxicity of carbon tetrachloride has probably been more extensively studied than that of any other hepatotoxic agent. Its toxicity has been studied both from the biochemical and pathological viewpoints, and therefore the data available provide particular insight into mechanisms of toxicity.

Carbon tetrachloride might be a simple molecule that, once administered to a several of animal species, causes centrilobular hepatic necrosis and fatty liver. Chronic treatment or exposure causes hepatic cirrhosis of the liver, liver tumors, and also renal damage. the clarification for the liver being the key target is that the toxicity of tetrachloromethane depends on metabolic initiation by CYP2E1 [Timbrel, 2009].

![Mechanism of hepatotoxicity of CCl₄](image)

Figure-2 : Mechanism of hepatotoxicity of CCl₄ Adapted from Timbrel (2009)

In spite of the fact that tetrachloromethane was initially thought to be evidence against metabolic assault, it's at present obvious that it's used by cytochromes P-450. The liver necrosis caused by CCl₄ is thought to be bio activated by CytochromP450 2E1 (CYP2E1), leading to the formation of trichloromethyl free radicals and reactive oxygen species (ROS), that start lipid peroxidation, oxidization of protein and damage the hepatocellular membrane [McCay et al., 1984]. This
method is followed by the release of inflammatory mediators from activated hepatic macrophages that are believed to potentiate the CCl4-induced liver injury [Kim et al., 2009; Raabe et al., 1998].

Adjustments within the action of CYP2E1 will affect the status to hepatic damage from CCl4. The trichloromethyl radical formed all through the metabolism of CCl4 is capable of binding to lipids, and this binding starts lipid peroxidation and liver damage [Ahmed et al., 2000].

1.3.5. Liver function tests

Estimation of the presence or absence of hepatic dysfunction is complicated by the large functional reserve of the liver and its power to regenerate rapidly. A diffuse minimal involvement of liver may produce more grossly abnormal laboratory test than a focal necrosis [Kaplan et al., 1988].

The following are the different biochemical parameters which are normally estimated to assess the liver function: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma–glutamyl transferase (GGT) and bilirubin total bilirubin, protein, [Klaassen, 2008; Nelson and Cox, 2005].

High levels of ALT is a more specific indicator of hepatic injury than that of AST, because high levels of AST may also be symbolic of muscle, heart or kidney injury [Jussi et al., 2006]. Elevated levels of ALP, GGT and bilirubin are indicative of compromised liver function and cholestasis [Larrey, 2002 ]

1.4. The kidney

The kidneys are a couple of organs situated in the back of the stomach area. Every kidney is around 4 or 5 inches long and contains around a million units called nephrons which unite to form collecting ducts or tubules, Each kidney is
supplied by a renal artery and every nephron is a microscopic filter for blood. [Mohan, 2010; Brenner, 2007]

![Figure-3: Structure of kidney](image)

1.4.1. Functions of the kidney

The kidney functions primarily as an organ for discharge of metabolic waste and maintenance of blood pH, fluid volume and electrolyte structure [Klaassen, 2008; Scanlon and Sanders, 2007]. The procedures involved in the evacuation of waste and maintenance of fluid composition and volume leads to the formation of urine [Klaassen, 2008].

The key unit of the kidney involved with these procedures is known as the nephron [Scanlon and Sanders, 2007]. The tubular nephron comprises of glomerular capsule, proximal convoluted tubule, descending loop of Henle, ascending loop of Henle and distal convoluted tubule [Van Der Graaff, 2001].

In forming urine, the kidneys change plasma into urine through filtration, reabsorption and secretion. Blood from flow enters the glomerulus where it is sifted and the filtrate moves into the Bowman’s capsule. As the filtrate moves from the Bowman’s capsule to the proximal convoluted tubule through to the distal convoluted tubule, salts and other molecules are reabsorbed through active transport, though water is reabsorbed
by osmosis, negative ions by passive diffusion and small proteins through pinocytosis are taken up by proximal tubule cells [Scanlon and Sanders, 2007; Saladin, 2003].

Tubular secretion happens from the blood into the filtrate in the renal tubule. Creatinine, amonia and other waste products are secreted into the filtrate, in this way, changes the composition of the tubular liquid though hydrogen ions are secreted to help keep up blood pH [Scanlon and Sanders, 2007; Saladin, 2003; Van De Graaff, 2001]. As the tubular liquid leaves the distal convoluted tubule into the collecting duct, it is called urine, which is then put away in the urinary bladder and later discharged [Saladin, 2003].

Other homeostatic function of the kidney include: detoxifying harms, activation of vitamin D, production of glucose, generation of erythropoietin, secretion of renin, controlling hypertension and pressure of oxygen and carbon dioxide of the blood [Scanlon and Sanders, 2007; Saladin, 2003].

1.4.2. Kidney Disease

Disorders of the kidney are different however individual with kidney illness with regularly show characteristics clinical features. Common clinical states of the kidney include [Tortora, 2005; Brenner, 2007]

Pyelonephritis (disease of kidney pelvis): Bacteria may contaminate the kidney, as a rule causing back ache and fever. A spread of microorganisms from an untreated bladder disease is the most well-known reason for pyelonephritis.

Glomerulonephritis: An overactive immune system may assaul The kidney, causing inflammation and some injury. Blood and protein in the urine are normal issues that happen with glomerulonephritis. It can likewise bring about kidney failure.
Nephrotic disorder: Damage to the kidneys makes them spill a lot of protein into the urine. Leg swelling (edema) might be a symptom.

Renal cysts: A benign emptied out space in the kidney. Separated kidney cysts happen in numerous normal individuals and never debilitate kidney function.

Kidney stones (nephrolithiasis): Minerals in urine aggregate to form crystals (stones), which may develop large enough to block urine flow. It's viewed as one of the most painful conditions. Most kidney stones pass individually yet some are too large and should be treated.

Acute kidney failure: A sudden decreases in renal function. Drying out, a blockage in the urinary tract or kidney harm can cause intense kidney failure, which might be reversible.

Chronic renal failure: A permanent partial loss of renal function. Diabetes and high blood pressure are the most well-known reason.

Polycystic kidney diseases: A hereditary condition bringing about vast growths in both kidneys that debilitate their capacity.

Kidney cancer.: Renal cell carcinoma is the most widely recognized tumor influencing the kidney. Smoking is the most widely recognized reason for kidney tumor.

Diabetic nephropathy: High glucose from diabetes logically harms the kidneys, in the end causing chronic kidney diseases. Protein in the urine(nephrotic disorder) may likewise come about.

Renal failure is basically determined by a decrease in glomerular filtration rate, the rate at which blood is filtered in the glomeruli of the kidney. This can be observed by a decrease or absence of urine production or determination of waste products (creatinine or urea) in the blood. The kidneys are affected by a variety
of chemicals. Man is exposed to medicines, industrial and \environmental chemicals and a variety of naturally occurring substances.[Tortora, 2005]

Nephrotoxicity is an adverse effect of specific antibiotics, anticancer agents and various synthetic agents. A few chemicals cause an acute injury and others may produce chronic renal damage that may lead to end stage renal failure and renal malignancies. Renal failures are mostly of two types - acute renal failure and chronic renal failure. Acute renal failure (ARF) is characterized with the aid of a reversible loss of kidney function and azotemia that advance quickly by few hours to days. Acute renal failure is frequently symptomatic. It can be detected through measuring the levels of creatinine and blood urea nitrogen in the blood. Chronic renal failure (CRF) is identified through increased irreversible deterioration of the kidneys because of moderate destruction of renal parenchyma. [Eugene, 2001]. An early change detected by means of pollutants at the kidney is the accumulation of intracellular calcium. This increase in calcium is located in plasma membrane, mitochondria, endoplasmic reticulum and additionally within the cytoplasm. Because of an increase in calcium the permeability of the internal membrane of the mitochondria is affected that modify the electrochemical gradient throughout it that decreases the oxidative phosphorylation capacity of the mitochondria. Disordered permeability leads to the lack of enzymes and nucleotides [Davison, 1988; Eugene, 2001].

In vivo and in vitro studies have demonstrated the impact of free radicals like superoxide hydroxyl ions and hydrogen peroxide which can be critical mediators of tissue damage. Free radical injury and oxidative stress were implicated in many renal diseases like acute renal failure, IgA nephropathy, anemia of chronic renal failure and ischemic kidney. Most threat assessment choices are based on records concerning aminoglycosides, halogenated anesthetic, and numerous heavy
metals in which an excellent concordance among animals and findings in people exposed to those agents.

1.4.3. Nephrotoxic Agents [Schrier, 1993]


1.4.4. Mechanisms of APAP induced renal toxicity

Acetaminophen is metabolized via conjugation with glucuronic acid and sulphate leading to excretion, before that a portion of APAP is metabolized by cytochrome P-450. Cytochrome P-450 oxidation of acetaminophen results in the production of a chemically reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which then reacts with glutathione (GSH) to form an APAP-GSH conjugate. Depletion of reduced glutathione (GSH) and lipid peroxidation plays a crucial role in the development. GSH has an important function as a cellular protective mechanism against toxic metabolites. At therapeutic doses, NAPQI is removed by GSH; however, at overdoses of APAP, the GSH is exhausted and the NAPQI then binds to cellular proteins, including a number of mitochondrial proteins, which leads to cause nephrotoxicity. In addition, NAPQI leads to mitochondria
dysfunction and reactive oxygen species (ROS) formation, which in turn leads to peroxy nitrite and tyrosine nitration. The events in the mechanism, affects mitochondrial permeability transition, which leads to disturbances in the permeability transition, which leads to disturbances in the permeability of the inner mitochondrial membrane [Mazer et al., 2008].

**Figure-4:** Mechanism of paracetamol induced Nephrotoxicity

### 1.4.5. Kidney function Test

Periodic examination of kidney function is a part of medical care for adults and chronically sick individuals [Stevens et al., 2006]. Blood urea nitrogen and creatinine are the commonly used biomarkers for the assessment of kidney injury. These are however not specific to the cause or location of the injury, hence, there is a great need for biomarker improvement [Goodsaid et al., 2009].

#### 1.4.5.1. Blood Urea Nitrogen (BUN)

Urea is the principal waste product of protein catabolism. It is produced in the liver from ammonia which is formed as a result of the deamination of amino acids. Urea is dissolved in the blood and is mainly excreted by the renal tubules and a small amount in the sweat. Blood urea nitrogen (BUN) test is usually done to assess the functioning of kidneys and monitor progression and treatment of kidney disease [Edgar, 2012; WebMD, 2012; Summer, 2012].
High levels of BUN in the system may be due to kidney disease, urinary tract obstruction, congestive heart failure and excessive protein levels in the gut (due to high protein diet). It may also be due to gastrointestinal bleeding, fever, metallic poisoning, pneumonia, administration of cortisol-like steroids and stressful situations [David, 2013; Summer, 2012] as well as increased protein catabolism, hypovolemia (dehydration) and antibiotics that enhance protein catabolism (e. g. tetracyclins). Whereas low levels of BUN may be due to liver disease or injury, low protein diet, malnutrition, hypervolemia (over-hydration), late pregnancy and amyloidosis [Deepak et al., 2007; Papadakis and Arief, 1987].

1.4.5.2. Creatinine levels

Creatinine is formed from creatinine phosphate, a high energy molecule used by skeletal muscle [Nelson and Cox, 2005]. Creatinine is a waste product of metabolism and thus, is not known to have any physiological function. The kidneys excrete creatinine, thus, measurement of creatinine levels is used to evaluate renal function and monitor progression and treatment of kidney disease. Levels of creatinine in the urine and serum can be used to monitor the creatinine clearance from the body which is an indicator of glomerular filtration rate. High levels of creatinine are found in renal dysfunction and reduced renal blood flow whiles decreases levels are found in muscular dystrophy [Klaassen, 2008; Nelson and Cox, 2005; Markus and Rima, 2000].

1.5. Oxidative stress assessment

Aerobic cells depend on oxygen as the final acceptor of electrons in respiration. This lets in them to extract more energy from food than might be feasible without oxygen and consequently producing reactive oxygen species (ROS) or free radical oxygen [Goodsell, 2007]. Free radicals and associated species are comprised
of oxygen (ROS) and nitrogen (reactive nitrogen species/ RNS). These are normally produced in the body from ordinary metabolism or from effect of outside sources like pollution, cigarette smoke, pressure, radiation and remedy, on the body [Sivanandham, 2011]. Examples of reactive species consist of: singlet oxygen, hydrogen peroxide, superoxide anion, nitroxides and hydroxyl radicals [Sarma et al., 2010].

Though ROS are produced by way of regular aerobic cells, their physiologic role depends on their levels in the cell [Weydert and Cullen, 2009]. The accumulation of these reactive species leads to oxidative strain [Sivanandham, 2011]. They are shown to have damaging impact on cellular parts and subsequently result in cell destruction and dysfunction [Gholamreza et al., 2005; Ezzat, 1996]. Several evidences also show that free radicals and decrease in antioxidant effect are implicated in xenobiotics – caused nephrotoxicity, rheumatoid arthritis, cataract, most cancers, artherosclerosis, getting older, cardiovascualr and neurodegenerative disorders [Aslam et al., 2013].

The human body utilizes several method (e.g. Decreased glutathione, superoxide dismutase, catalase and others) for counteracting oxidative stress [Aslam et al., 2013; Sivanandham, 2011].

1.5.1. Reduced glutathione

It is a significant part of cells throughout the body for cellular maintenance and survival [Main et al., 2012]. GSH has a role in detoxification of varied compounds in reactions involving glutathione peroxidases (GPx) and glutathione-S-transferases (GST) [Johnson et al., 2012].

When glutathione is oxidized, glutathione reductase uses NADPH as a co-factor to convert the oxidized glutathione (glutathione disulphide, GSSG) to its
reduced form (GSH). The ratio of GSH to GSSG in a cell or tissue is used to assess toxicity within the cell or tissue [Johnson et al., 2012; Townsend et al., 2003]. Insufficient amounts of GSH within a cell predispose the cell to damage by oxidative stress [Aslam et al., 2013]. An inadequate amount of GSH in tissues has been implicated in various disease conditions including: renal injury, carcinogenesis, liver damage, pancreatitis and neurodegenerative diseases [Johnson et al., 2012; Townsend et al., 2003].

1.5.2. Superoxide dismutase (SOD)

It has been shown that superoxide radicals are implicated in various disease conditions [Christos et al., 2008]. Superoxide ion is produced from different sources like normal cellular respiration, activated polymorphonuclear cells, mitochondria electron flux and endothelial cells [Lenaz, 2001; McCord and Omar, 1993].

SOD constitutes the first line of defence against reactive oxygen species [Abreu and Cabelli, 2009; Larry and Garry, 1979]. Superoxide dismutase (SOD) is believed to be absent in most obligate anaerobes but existent in all aerobic cells. This may be due to its physiological role in providing defence against potential damage by superoxide radical produced from aerobic metabolic reaction. Research has established that low levels of superoxide dismutase have been found in tissues prone to oxidative stress [Stevens et al., 2000; Larry and Garry, 1979].

1.5.3. Catalase

Hydrogen peroxide is produced from a number of oxidative reactions in the cell. It however has a detrimental effect on cellular structures like proteins, lipids and DNA and therefore should be quickly removed from the body. To this effect, catalase is frequently used by cells to convert hydrogen peroxide to less
reactive products – oxygen and water [Sivanandham, 2011; Weydert and Cullen, 2009]. It is found in most living cells but abundant in liver, kidney and blood [Klaassen, 2008]. Catalase also utilizes peroxide to detoxify various metabolites and toxins like alcohol, formaldehyde, phenols, acetaldehyde and formic acid [Sivanandham, 2011; Klaassen, 2008]. Several studies have shown that catalase levels are low during hepato-renal injury [Adejuwon et al., 2014],

1.5.4. Lipid peroxidation

Lipid peroxidation is a natural metabolic process that takes place in both plants and animals (Repetto et al., 2012; Janero, 1990). In the process of lipid peroxidation, reactive species attract electrons from lipids of the cell membranes, leading to cell damage and eventually cell death [Gago – Dominguez et al., 2007; Girotti, 1998]. Products of lipid peroxidation (e.g. alcohols, ketones, aldehydes, alkanes and esters) are known to have various roles in the signal transduction cascade, the control of cell proliferation, and the induction of differentiation, maturation and apoptosis [Barrera et al., 2004; Cejas et al., 2004].

However in pathological situations, the reactive oxygen and nitrogen species are generated at higher than normal rates, and as a consequent, there is increased destruction of cellular structures and death of cells [Repetto et al., 2012; Barrera et al., 2004]. The quantification of lipid peroxidation is therefore necessary to assess the role of oxidative injury in pathophysiological conditions [Repetto et al., 2012]. Thus, lipid peroxidation is assessed in relation to the amount of malondialdehyde (MDA) produced. Malondialdehyde is a byproduct from the peroxidation of lipids, it combines with several functional groups on molecules including lipoproteins, DNA and proteins and it helps to visualize the MDA – adduct formed [Janero, 1990].
Treatment option for hepatotoxicity and common liver diseases such as cirrhosis, fatty liver and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine and corticosteroid are inconsistent at best and the incidence of side effects is profound, though the treatment is worse than the disease [Scott et al., 1998].

Nephrotoxicity is a common and important side effect of various drugs and this limits their clinical relevance and is a critical complication of therapeutics [Rajitha et al., 2013].

Conventional drugs used in the treatment of liver and kidney diseases are sometimes inadequate and can lead to serious side effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver and kidney diseases in order to replace currently used drugs of doubtful efficacy and safety. Plant extracts and phytochemicals are very beneficial in the treatment of various diseases and conditions (Varalakshmi et al., 2011). In view of the paramount importance of herbal drug for liver diseases and kidney disorder, we made a scientific approach to explore the Hepatoprotective and Nephroprotective potential of Combretum albidum and Salacia fruticosa a well-known plant in Indian traditional medicine.
Aim and Objective

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Drug discovery programme for new therapeutic entity is based on natural 'Lead'. So, the present aim of the study is to evaluate the hepatoprotective, nephroprotective and antioxidant potential of *Combretum albidum* whole plant and *Salacia fruticosa* root extracts in traditional medicine as well as to identify and characterize the active constituents of both medicinal plants.

Objective

- To prepare the extract of the selected plants.
- To identify the different phytoconstituent present in the plants extract by qualitative analysis
- To characterize phytochemical present in the plants extract by GC/MS/MS analysis
- To study the acute toxicity of the plants extract.
- To assess the hepatoprotective potential of plants extracts against CCl4-induced liver injury in rats (in vivo).
- To evaluate the nephroprotective effect of the plants extracts against Acetaminophen (APAP)-induced nephrotoxicity in rats (in vivo).
- To determine the antioxidant properties of the plants extracts in rats liver and kidney homogenate (in vivo).