CHAPTER 1

INTRODUCTION

The history of herbal medicine is as old as human civilization. Medicinal plants have been utilized as a constant source of medicaments for the variety of diseases. The plants are known to provide a rich source of botanical anthelmintics, antibacterials, and insecticides (Duke, 2002).

Compounds and extracts derived from the Mother Nature's diversity have found uses in medicine i.e allopatic, homeopathy and Ayurveda, agriculture, beauty products, and health products in ancient and modern societies around the world. Therefore, the visionary to access natural products, understanding their usefulness and derivation applications has been a major driving force in the field of natural product research (Cannell, 1998; Chopra, 1993; Ahmad and Beg, 2001; Kirtikar et al., 1918; Ali et al., 2008).

Natural products generally belong to any of the following category:

1. An entire organism which could be a plant, an animal, or a microorganism that has not been subjected to any kind of processing or treatment other than a simple process of preservation (e.g., drying)

2. Part of an organism which could be leaves, roots, steam, bark, flowers of a plant, and an isolated organ from an animal

3. The wholesome compound which could be an alkaloid, sugars, coumarin, glycosides, lignin, steroids, flavonoids, terpenoids, is
isolated from plants, animals or microorganisms (Samuelsson, G. 1999).

1.1 KIDNEY

The kidneys are organs that serve several vital regulatory roles in most animals, including vertebrates and some invertebrates. Kidneys are pair of organs found in the abdominal cavity along posterior muscular wall of the human body. Kidneys are called as retroperitoneal organs, since they are behind the peritoneum line, which separates the chest from abdomen. Perirenal fat or adipose tissue which surrounds the kidneys serves as protective padding. Each kidney is about 4 or 5 inches long and is bean shaped. The left kidney is more superior to the right kidney because of the larger size of the liver on the right side of the body. These paired organs are normally located between the transverse processes of T12-L3 vertebrae. The width of the kidney is approximately 5 – 7.5 cm, whose length is about 11 – 12 cm, and with 2.5 cm of minimum thickness. In average human kidney varies from 125 – 170 g and 115 – 155 g for men and women respectively.

1.1.1 Structure of Kidney

The three major regions of kidney are the renal cortex, the renal medulla, and the renal pelvis. Kidneys are covered by tough capsule of fibrous connective tissue called renal capsule. Deep inside the renal capsule is the dense and soft vascular structure called renal cortex. Renal medullas are deep to the renal cortex and are seven cone shaped renal pyramids.

These pyramids are facing upwards towards the renal cortex, whose apexes pointing inward toward the center of the kidney and are aligned to their bases. Each apex connects to minor calyx, which are merged to form major calyces.
and three of them are further merged to form hollow renal pelvis at the center of the kidney. This renal pelvis exits the kidney to drain the urine into the ureter at the renal hilus. The structure of kidney with renal cortex, renal medulla and renal pelvis are shown in figure 1.

![Transverse structure of human kidney](image)

**Figure 1: Transverse structure of human kidney**

1.1.2 **The Nephron**

Each kidney has around million units called nephrons, each of which is a microscopic functional unit that filter blood to produce urine. This is made up of two parts: the renal corpuscle and renal tubule. Renal corpuscle is responsible for filtering the blood and is formed by the bundled network of capillaries of glomerulus and the glomerular capsule otherwise called as Bowman’s capsule. The outer layer of Bowman’s capsule holds the urine separated from the blood inside the capsule.
The mouth of the renal tubule is located at the far end of the glomerular capsule, opposite to the glomerulus.

The renal tubule concentrates urine and recovers non-waste solutes from the urine. This tubule is the one that carries urine to the renal pelvis from the glomerular capsule.

i. The curvy section of the renal tubule is called the proximal convoluted tubule, whose tubule cells reabsorb a large amount of the water and nutrients initially filtered into the urine.

ii. Loop of Henle is a long straight tubule that carries urine into the renal medulla before making a hairpin turn and returning to the renal cortex.

iii. Following the loop of Henle is the distal convoluted tubule.

iv. Finally, urine from the distal convoluted tubules of several nephrons go through the collecting duct, which carries the concentrated urine through the renal medulla and into the renal pelvis.

From the renal pelvis urine from many collecting ducts combines and flows out of the kidneys and into the ureters

1.1.3 Functions of Kidney

The role of the kidney is to filter the blood. The blood passes through the kidneys several times a day. They remove wastes, regulate the balance of electrolytes and control the body’s fluid balance. Following are the major functionalities of the kidney:
i. Filtration and excretion of metabolic waste products (urea and ammonium)

ii. Regulation of necessary electrolytes, fluid, and acid-base balance

iii. Stimulation of red blood cell production,

iv. Regulate blood pressure via the renin-angiotensin-aldosterone system,

v. Controlling re-absorption of water and maintaining intravascular volume

vi. Reabsorb glucose and amino acids and have hormonal functions via erythropoietin, calcitriol, and vitamin D activation that help bones stay strong.

1.1.4 Kidney Conditions

Following are the several kidney conditions that need attention:

i. **Pyelonephritis** (infection of kidney pelvis): Bacteria may infect the kidney and the spread of same from an untreated bladder infection will result in pyelonephritis causing back pain and fever.

ii. **Glomerulonephritis**: An overactive immune system may attack the kidney, causing inflammation and some damage leading to over filtering of blood and protein in the urine. It may also lead to kidney failure.

iii. **Kidney stones (nephrolithiasis)**: Formation of crystals (stones) due to the presence of minerals in urine, which may grow large enough to block urine flow. Most of the kidney stones pass on their own but
some are too large and require treatment. This is one of the most painful conditions that may occur.

iv. **Nephrotic syndrome:** Large amounts of protein will spill into the urine due to the damage of the kidneys. The symptom will be the swelling of legs (edema).

v. **Polycystic kidney disease:** A genetic condition resulting in large cysts in both kidneys that impair their function.

vi. **Acute renal failure (kidney failure):** A sudden worsening in kidney function. Dehydration, a blockage in the urinary tract, or kidney damage can cause acute renal failure, which may be reversible.

vii. **Chronic renal failure:** A permanent partial loss of kidney function. Diabetes and high blood pressure are the most common causes.

viii. **End stage renal disease (ESRD):** Complete loss of kidney function, usually due to progressive chronic kidney disease. People with ESRD require regular dialysis for survival.

ix. **Papillary necrosis:** Severe damage to the kidneys can cause chunks of kidney tissue to break off internally and clog the kidneys. If untreated, the resulting damage can lead to total kidney failure.

x. **Diabetic nephropathy:** High blood sugar from diabetes progressively damages the kidneys, eventually causing chronic kidney disease. Protein in the urine (nephrotic syndrome) may also result.

xi. **Hypertensive nephropathy:** Kidney damage caused by high blood pressure. Chronic renal failure may eventually result.
xii. **Kidney cancer:** Renal cell carcinoma is the most common cancer affecting the kidney. Smoking is the most common cause of kidney cancer.

xiii. **Interstitial nephritis:** Inflammation of the connective tissue inside the kidney, often causing acute renal failure. Allergic reactions and **drug side effects** are the usual causes.

xiv. **Minimal change disease:** A form of nephrotic syndrome in which kidney cells look almost normal under the microscope. The disease can cause significant leg swelling (edema). Steroids are used to treat minimal change disease.

xv. **Nephrogenic diabetes insipidus:** The kidneys lose the ability to concentrate the urine, usually **due to a drug reaction.** Although it's rarely dangerous, diabetes insipidus causes constant thirst and frequent urination.

xvi. **Renal cyst:** A benign hollowed-out space in the kidney. Isolated kidney cysts occur in many normal people and almost never impair kidney function.

### 1.2 LIVER

The liver has a pivotal role in human metabolism. The liver produces and secretes bile (to be stored in the gallbladder until needed) that is used to break down and digest fatty acids. It produces prothrombin and fibrinogen, both blood-clotting factors and heparin mucopolysaccharide sulfuric acid ester that helps keep blood from clotting within the circulatory system. It converts sugar into glycogen, which it stores until the muscles need energy, when it is secreted into the blood stream as glucose. The liver synthesizes proteins and cholesterol and converts carbohydrates
and proteins into fats, which are stored for later use. Blood proteins and hundreds of enzymes needed for digestion and other bodily functions are produced by the liver. The liver produces urea, while breaking down proteins, which it synthesizes from carbon dioxide and ammonia. Urea is eventually excreted by the kidneys. It also stores critical trace elements such as iron and copper, as well as Vitamins A, D and $B_{12}$.

The liver is also responsible for detoxifying the body of poisonous substances by transforming and removing toxins and wastes. There are five main sources of body toxins and wastes that the liver deals with: toxins from food (traces of pesticides and preservatives) and alcohol toxins from outside (drugs, adulterants and environmental pollutants); internally produced chemicals such as hormones, which are no longer needed; nitrogen containing waste left over from protein reuse; and energy production. These toxins and wastes are converted into less harmful substances by the liver and then eliminated from the body.

1.3 HEPATOTOXICITY

Liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory and neoplastic insults. In some instances the disease process is primary due to some of the most common diseases in humans, such as cardiac decompensation, disseminated cancer, and alcoholism and extra hepatic infection (George et al., 1997). Liver injuries may be viral or caused by drugs, chemicals and alcohol.

1.3.1 Liver Injury Due to Virus

Viral liver disease remains a common and challenging problem. Among the many diseases that can affect the liver, the most common is hepatitis infection.
Hepatitis can be caused by drugs, viruses, bacteria, mushrooms etc. The most common hepatitis viruses affecting the liver are hepatitis - A, hepatitis- B, hepatitis - C, hepatitis -D, and hepatitis -E (Zignego and Brechot, 1999; Kim, 2002; Penin et al., 2004; Strader et al., 2004). Among these Hepatitis-C and B are truly serious diseases with no known effective treatment (Banker, 2003; Pawlosky, 2004; Vandalli et al., 2004). Hepatitis-C will become an increasingly important cause of morbidity and mortality for HIV infected persons (David Macdougall, 2000).

### 1.3.2 Drug Induced hepatotoxicity

Drugs may affect the liver. The toxic chemicals such as antibiotics, chemotherapeutics, peroxidised oil, aflatoxins, carbon tetrachloride, chlorinated hydrocarbon acetaminophen and excess consumption of alcohol cause liver injury.

Most of the chemicals and drugs damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver (Dianzani et al., 1991). Drugs affect liver function by stimulating the activity of microsomal enzymes, a process known as enzyme induction. Enzyme induction may be important in determining the degree of hepatotoxicity (Conney, 1967).

Chemical drugs are important cause of chronic hepatic disease. Therapeutic agents have been held responsible for instances of chronic hepatitis, fatty liver and cirrhosis as well as several vascular and neoplastic lesions of the liver (Dixon et al., 1971; Scheuer, 1980).

### 1.3.3 Paracetamol (Acetaminophen) induced toxicity

Paracetamol (Acetaminophen), acetyl-para-aminophenol (APAP), a p-aminophenol derivative is an active metabolite of both acetanilide and phenacetin.
Acetanilide, the parent compound was first introduced as an antipyretic and analgesic in 1886 but its use was limited because of its toxicity. Consequently other p-aminophenol derivatives were tested which led to the introduction of phenacetin in 1887 followed by acetaminophen in 1893, which was first used in medicine by von Mering (Insel, 1990).

Acetaminophen (APAP) has been available in the United States since 1960 as an analgesic or antipyretic drug. Since then, APAP has become the most widely-used analgesic in the United States (Paulose-Ram et al., 2005). APAP is one of the most widely and commonly used drugs commonly used for the relief of fever and headaches due to its antipyretic and analgesic properties, and is a major ingredient in cold and flu remedies.

Though APAP is generally considered safe for human use at recommended doses, potentially fatal liver damages occurred in rare cases when an acute over-dose or even a normal dose was taken. Accordingly, APAP overdose is one the most common causes of drug poisoning world-wide.

Excessive use of APAP can cause multiple organ damages, especially of liver and kidney (Bertolini, 2006; Yapar et al., 2007). Other tissues have also been shown to be affected by acetaminophen, for instance eye (Zhao et al., 1997), lung (Hart et al., 1998), testes (Boyd, 1970), heart (Prescott, 1980) and lymphoid tissues (Cohen et al., 1997).

Acetaminophen toxicity causes liver injury and may result in liver failure, metabolic acidosis and central nervous system depression which occurred early after an acute acetaminophen overdose and in the absence of manifest liver failure (Brett Roth et al., 1999). Moreover, it was reported that even borderline high
doses are hepatotoxic for some infants (Heubi et al., 1998). In addition, APAP has been found to cause tubular necrosis, pancreatitis, and myocardial necrosis (Prescott, 1980).

1.3.4 Clinical stages of acetaminophen overdose

In adults, the usual dosage of acetaminophen is 335 to 650 mg every 4 to 6 hours or 1000 mg 3 or 4 times a day, for a total no greater than 4 g/d (Tisdale, 2010). Acetaminophen is rapidly and almost completely absorbed from the gastrointestinal tract. After oral administration of immediate or extended-release acetaminophen preparations in therapeutic doses, peak plasma or serum concentrations occur within 1 to 2 hours, respectively. After ingestion of high dose of an immediate-release preparation, absorption is delayed but invariably is complete within 4 hours (Lewis and Paloucek, 1991; Albert et al., 1974; Zarro, 1987). The clinical course of acetaminophen overdose has 4 stages (Anker and Smilkstien, 1994).

First stage occurs from the time of ingestion to 24 hours after ingestion. The patient typically has anorexia, nausea, vomiting, and diaphoresis. The results of laboratory tests are usually normal. However, the level of transaminases may be slightly increased.

Stage two occurs after 24 to 72 hours of ingestion. The patient may actually appear to have improved clinically, but results of laboratory tests begin to be abnormal. Abnormalities include increases in serum aspartate aminotransferase and alanine aminotransferase activity, an increase in the serum level of bilirubin, and
a prolonged prothrombin time. During this stage, patients may also complain of pain in the right upper quadrant of the abdomen (Lewis and Paloucek, 1991).

Stage three occurs after 72 to 96 hours of ingestion and is also known as the hepatic stage. The abnormal results of laboratory tests hit the highest point during this stage. Aspartate aminotransferase activity may be as great as 10,000 U/L, and alanine aminotransferase activity may be greater than 1000 U/L (Lewis and Paloucek, 1991). Hepatotoxic effects become evident when features such as jaundice, gastrointestinal bleeding, coagulopathy, hypoglycemia, renal failure, and abnormal electrolyte levels are apparent. Death, although infrequent, may result from fulminant hepatic failure due to hepatic encephalopathy or hepatorenal syndrome.

Final stage is the recovery stage of acetaminophen overdose and usually occurs 7 to 8 days after ingestion. In patients who recover, signs and symptoms resolve, and results of laboratory tests return to normal.

1.3.5 Heptotoxicity and drug metabolism

The biotransformation of lipophilic compounds into water-soluble derivatives that are more readily excreted is a physiological role of the liver. The liver receives more than 80% of its blood flow from the gastrointestinal tract and has high capacity for both Phase I and Phase II biotransformations. Cytochrome P450 enzymes play a primary role in the metabolism of an incredibly diverse range of foreign compounds including therapeutic agents. Such compounds may concentrate in the liver by various processes including active transport systems.
Although the major role of drug metabolism is detoxification, it can also act as an intoxication process. Thus, foreign compounds can undergo biotransformation to metabolites that have intrinsic chemical reactivity toward cellular macromolecules. The propensity of a molecule to form such chemically and structural alerts are now well defined.

![Drug metabolism and toxicity](image)

**Figure 2: Drug metabolism and toxicity (Srivastava et al., 2010)**

The versatility of P450 together with the reactivity of their oxygen intermediates enables them to functionalize even relatively inert substances to the direct formation of diverse chemically reactive species. Such metabolites are short-lived with half-lives of generally less than one minute and are not usually detectable in plasma. Their intracellular formation can be inferred from endogeneous trapping reactions or physio-chemical techniques and may be modulated by enzymes induction, enzyme inhibition and gene deletion. The process of drug metabolism and toxicity is depicted in figure 2.
Many chemicals undergo bioactivation in the liver but are not hepatotoxic. The best example is the lack of hepatotoxicity with therapeutic doses of acetaminophen. Tight coupling of bioactivation with bioinactivation may be a one reason for this. Many enzymatic and non enzymatic pathways of bioinactivation are present in the liver, which is perhaps the best quipped of all the organs in the body to deal with toxins.

Typical examples of bioinactivation pathways include GSH conjugation of quinines by GST and hydration of arene oxides to dihydrodiols by epoxide hydrolases. It is only when a reactive metabolite is a poor substrate for such enzymes that it can escape bioinactivation and thereby damage proteins and nucleic acids. Moreover covalent binding per sec does not necessarily lead to drug hepatotoxicity.

1.3.6 **Biochemical mechanisms of acetaminophen-induced liver cell death**

A fraction of the dose of APAP is metabolically activated to a reactive metabolite (NAPQI), which first depletes cellular glutathione and subsequently covalently binds to cellular proteins. These initiating events lead to disturbances of the cellular \( \text{Ca}^{2+} \) homeostasis, with increase of the cytosolic \( \text{Ca}^{2+} \) levels, Bax and Bid translocation to the mitochondria, and a mitochondrial oxidant stress and peroxynitrite formation. The Bcl-2 family members form pores in the outer mitochondrial membrane and release cytochrome-C, Smac, Apoptosis inducing factor (AIF), and endonuclease G from the mitochondrial intermembrane space.
Figure 3: Mechanism of APAP induced cell necrosis

Reactive oxygen species and peroxynitrite induce the Mitocondrial membrane permeability transition (MPT), which causes the collapse of the mitochondrial membrane potential, eliminates ATP synthesis, and causes further release of mitochondrial proteins as shown in figure 3 (Rang et al., 2014). The declining ATP levels appear to prevent caspase activation by the release of cytochrome c and Smac. AIF and endonuclease G translocate to the nucleus and
induce DNA fragmentation, which is further aggravated by the nuclear Ca\(^{2+}/\text{Mg}^{2+}\) dependent endonuclease DNAS1L3.

The massive nuclear DNA damage and the rapid elimination of functional mitochondria, together with activation of intracellular proteases (calpains), lead to cell membrane failure and oncotic necrosis of the hepatocytes.

The postulated intracellular signaling events after APAP overdose can explain the massive cell death and liver failure. However, many aspects are still unclear and require further investigation. In addition, it has to be kept in mind that APAP-induced cell death \textit{invivo} can be modulated by changes in the expression levels of P450 and phase II detoxification enzymes, variation in the GSH and antioxidant levels (nutritional status), and preexisting conditions affecting the susceptibility of hepatocytes (steatosis, mitochondrial abnormalities, inflammation). Therefore, to most effectively protect against APAP overdose, it is important to focus on central mechanisms of the pathophysiology.

1.4 LIPID PEROXIDATION

The administration of APAP to rats exerts hepatotoxic effects through lipid peroxidation. Lipid peroxidation is an oxidative destruction of lipid containing any number of carbon-carbon double bonds. Free radical is an atom or molecule that contains one or more unpaired electrons in the outer most orbits capable of independent existence (Delmastro, 1980). Lipid peroxidation is initiated by free radicals that have sufficient reactivity for the uptake of a hydrogen atom from unsaturated lipids (Halliwell and Gutteridge, 1985). After this, rearrangement of double bonds in unsaturated fatty acids takes place, thus producing a variety of breakdown products such as schiff’s bases, alcohols, ketones, aldehyde fragments,
especially malondialdehyde and a mixture of fluorescent products (Gardner, 1975; Cohen, 1979).

1.4.1 Oxidative Stress

Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative stresses in liver (Handa et al., 1989). Oxidative stress is considered to play a prominent causative role in many diseases including liver damage (Kiso et al., 1984). Oxidative stress is the state of imbalance between the levels of defense system and production of oxygen derived species. Increased oxygen concentration and production of oxygen-derived species such as superoxide radical (O$_2^-$), hydroxyl radical (OH) and hydrogen peroxide cause Oxidative stress (Zhu et al., 2004).

Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage and DNA strand breaking (Ghosal et al., 1996). Several anti-inflammatory, digestive, antinecrotic, nephroprotective, neuroprotective, and hepatoprotective drugs have recently been shown to have antioxidant and/or radical scavenging mechanism as part of their activity (Lin and Huang, 2000; Repetto and Llesuy, 2002).

An over dose of APAP can cause overproduction of ROS during formation of N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 (Dahlin et al., 1984). This mechanism has been suggested to participate in the development of oxidative stress and toxicity in APAP-induced hepato-renal disorders (James et al., 2003).
Oxidative stress is another mechanism that has been postulated to be important in the development of acetaminophen toxicity. Thus, increased formation of superoxide would lead to hydrogen peroxide and peroxidation reactions by Fenton-type mechanisms. It has been shown that NAPQI reacts very rapidly with GSH (Coles et al., 1988), and there are a number of potential mechanisms that have been suggested to play a role.

Under conditions of NAPQI formation following toxic acetaminophen doses, GSH concentrations may be very low in the centrilobular cells, and the major peroxide detoxification enzyme, GSH peroxidase, which functions very inefficiently under conditions of GSH depletion (Nakamura et al., 1974), is expected to be inhibited. In addition, during formation of NAPQI by cytochrome P450, the superoxide anion is formed, with dismutation leading to hydrogen peroxide formation (Dai and Cederbaum, 1995).

1.4.2 The peroxidation process

Lipid peroxidation is a chain reaction, which is initiated by the attack of free radicals on the membrane lipids that are capable of absorbing a hydrogen atom from the methylene group; this is known as initiation phase (Halliwell and Gutteridge, 1985). The carbon radicals thus formed are stabilized by molecular rearrangements to produce a conjugated diene, which easily reacts with an oxygen molecule to give a peroxyl radical (Mailard et al., 1983; Esterbauer et al., 1991). The peroxyl radicals can further abstract a hydrogen atom from another lipid molecule to form lipid peroxide; this is the propagation stage of lipid peroxidation (Halliwell and Gutteridge, 1985).
Alternatively, the peroxy radicals can form cyclic peroxide and cyclic endo peroxide, which on fragmentation leads to the formation of a cytotoxic aldehyde like malondialdehyde (Pryor et al., 1976; Gutteridge et al., 1984; Wade and Van Rij, 1988). Once started, lipid per oxidation proceeds as a chain reaction until the PUFA substrate is consumed or until the radicals self annihilate, is called termination phase (Halliwell and Gutteridge, 1990).

1.4.3 Antioxidants

Any agent that prevents the tissue injury of any free radical catalyzed by radical production is an antioxidant.

“Antioxidants are a type of complex compounds found in our diet that act as a protective shield for our body against certain diseases such as arterial and cardiac diseases, arthritis, hepatorenal disease, cataracts and also premature ageing along with several chronic diseases”.

The above definition gives an idea about what actually an antioxidant is. Still a lot of work has to be carried out on getting exact information about antioxidants, their exact amount in one’s diet and their function.

Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA (Fang et al., 2002). They may be low molecular weight molecules, therapeutic agents; enzymatic agents that dismutate, scavenge partially reduced oxygen, chelate transition metal, oxidant interconversion and prevent cellular components against oxidative degeneration.
Antioxidants can be classified into two major classes *i.e.*, enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources (Lee *et al.*, 2004).

Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability.

Reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signalling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase, as a result of over-production of ROS, due to the exposure to external oxidant substances or a failure of enzyme regulatory mechanisms leading to damage of cell structures, DNA, lipids and proteins (Valko and Mazur, 2006).

α-Tocopherol (Vitamin E) is an essential nutrient which functions as a chain breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (Vitamin C) is also part of the normal protecting mechanism. Other non-enzymatic antioxidants include carotenoids, flavonoids, and related polyphenols, α-lipoic acid, glutathione etc.
1.4.4 Levels of Antioxidant Action

Antioxidants capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase (SOD) that catalyses the dismutation of superoxide to $\text{H}_2\text{O}_2$ and catalase that breaks it down to water (Sies, 1997; Cadenas and Packer, 1996). Interception of free radicals is mainly by radical scavenging, while at the secondary level scavenging of peroxyl radicals are affected. The effectors include various antioxidants like vitamin C and E, glutathione other thiol compounds, Carotenoids, flavonoids, etc. at the repair and reconstitution level, although mainly repair enzymes are involved (Sies, 1997; Cadenas and Packer, 1996).

1.5 NEPHROTOXICITY

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals. It is well established that toxic nephropathies are not restricted to a single type of renal injury. Some chemicals target one discrete anatomical region of the kidney and may affect only one cell type. Chemical insult to the kidney may result in a spectrum of nephropathies that are indistinguishable from those that do not have a chemical etiology.

1.5.1 Acetaminophen induced nephrotoxicity

Large doses of acetoaminophen can produce acute proximal tubular necrosis, especially in male Fischer-344 rats (Newton et al., 1983a,b). Nephrotoxic dosages of paracetamol bind covalently to renal protein (Nelson, 2003) by an NADPH-dependent, cytochrome-P-450-mediated process (Newton et al., 1983 a,b).
Alternatively, paracetamol is enzymatically deacetylated to para-aminophenol, a potent selective nephrotoxin that damages the proximal tubule (Calder et al., 1979). Para-aminophenol produces acute necrosis of the proximal convoluted tubules in rats after a single injection (Green et al., 1969), and has been demonstrated to be a minor metabolite of paracetamol in the Fischer-344 rat and its isolated perfused kidney (Newton et al., 1982).

Paracetamol (N-acetyl-p-aminophenol) is structurally closely related to para-aminophenol, and metabolites have been shown to be excreted by the biliary route in rats (Siegers and Klaassen, 1984) and mice (Fischer et al., 1985). These metabolites are the glucuronic acid and sulfate conjugates (Siegers and Klaassen, 1984) and the glutathione conjugate. Toxicity arising from para-aminophenol has been previously suggested to result from a dose-related depletion of kidney reduced glutathione and covalent binding to essential renal macromolecules (Crowe et al., 1977).

The pathophysiology of renal toxicity in acetaminophen poisoning has been attributed to cytochrome P450 mixed function oxidase isoenzymes present in the kidney, although other mechanisms have been elucidated, including the role of prostaglandin synthetase and n-deacetylase enzymes. Paradoxically, glutathione is considered as an important element in the detoxification of acetaminophen and its metabolites; however, its conjugates have been implicated in the formation of nephrotoxic compounds. Acetaminophen-induced renal failure becomes evident after hepatotoxicity in most cases, but can be differentiated from the hepatorenal syndrome, which may complicate fulminant hepatic failure.
1.5.2 Biochemical mechanism of acetaminophen-induced nephrotoxicity

The primary toxicity of acetaminophen is the result of drug metabolism in both the liver and extrahepatic tissues (Gu et al., 2005). Only 1% of the drug is excreted unchanged in the urine. With therapeutic dosing in adults, approximately 63% of acetaminophen is metabolized via glucuronidation and 34% by sulfation. These phase II reactions occur primarily in the liver and result in water-soluble metabolites that are excreted via the kidney. At therapeutic doses, 5% percent of APAP is oxidized by the microsomal P-450 enzyme system to a reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI). In therapeutic dosing, this electrophilic metabolite is then reduced by glutathione and subsequently excreted as mercapturic acid, a relatively benign compound. In the setting of excess APAP, stores of sulfate and glutathione are depleted. This shunts more of the acetaminophen to the CYP-450 mixed function oxidase system, generating more NAPQI reactive intermediates.

When large doses of drug are ingested, there is more severe glutathione depletion as well as massive production of metabolites, which compounds the toxicity, leaving large amounts of reactive species unbound. These electrophilic intermediates then form adducts with sulfhydryl and glutathione moieties on cellular proteins (Bessems et al., 2001). This process disrupts homeostasis, with subsequent activation of caspases and lysosomal enzymes that initiate apoptosis, or programmed cell death. This has been demonstrated in both liver and kidney tissue in animal models. The resultant cell death leads to tissue necrosis and ultimately organ dysfunction (Khandkar et al., 1991; Lorz et al., 2005). The mechanism of acetaminophen toxicity is well described in the liver, but is less clearly understood in the kidney. There are several potential mechanisms of renal toxicity based on both animal and human data. Possible mechanisms include the cytochrome P450
pathway, as well as prostaglandin synthetase, and N-deacetylase enzymes (Bessems et al., 2001).

The CYP-450 microsomal enzymes involved in this process are found in both the liver and kidney, although they differ somewhat in each organ. The severity of renal damage and the quantity of reactive adducts in tissues can be significantly reduced when the CYP-450 inhibitor piperonyl butoxide is administered (Bessems et al., 2001). In addition, it has been noted that conditions that are associated with increased activity of the CYP-450 system enhance acetaminophen toxicity. Examples include chronic alcohol use and ingestion of drugs that induce these enzymes, such as anticonvulsants (Bray et al., 1992). The CYP-450 isoenzyme that is primarily involved in the biotransformation in the kidney is CYP 2E1, which is inducible by testosterone.

1.5.3 Hydrogen peroxide (H$_2$O$_2$) induced nephrotoxicity

There has been considerable interest in recent years in the damage that can be done to living systems by generation of reactive oxygen species, such as H$_2$O$_2$, and hydroxyl radical (‘OH). It has been proposed that much of the toxicity of H$_2$O$_2$ to living organisms is due to the iron ion-dependent generation of ‘OH, and/or other powerful oxidants.

Hydrogen peroxide (H$_2$O$_2$), a diffusible reactive oxygen metabolite formed by either enzyme catalyzed or spontaneous dismutation of superoxide anion, is implicated in the pathogenesis of tissue damage in glycerol-induced injury, ischemia-reperfusion injury, gentamicin nephropathy and neutrophil-dependent glomerular injury. H$_2$O$_2$ is a potential source for HO•, one of the most dangerous radicals HO•, through the Fenton reaction in the presence of transition metal ions.
Oxidative stress may affect cell proliferation, differentiation, and survival by activating signaling pathways. However, prolonged or high levels of oxidative stress cause neurotoxicity and neuronal cell death in neurodegenerative diseases and aging. Oxygen free radicals have been implicated in several biological processes potentially important in glomerular diseases (Shah 1984, Shah 1987) and also their role in neutrophil mediated glomerular diseases (Rehan 1985, Rehan 1986). It is well known that H₂O₂-induced oxidative stress disrupts MMP mitochondrial membrane potential and results in mitochondrial

H₂O₂ will cross the cell membrane and attack at different places by converting into OH•. H₂O₂ generates free OH• radicals in presence of transition metal ions (Halliwell, 1978). Metal ions are essential as enzyme cofactor and prosthetic groups. A redox-active transition metal mediates oxidation reduction reaction through reversible changes of metal ion.

H₂O₂ produces hydroxyl radicals (OH•) in the presence of Fe²⁺ ions known as superoxide driven Haber Weiss reaction. Superoxide O₂•⁻ meets many fates and finally gets converted to H₂O₂. These free radicals react with membrane bound polyunsaturated fatty acids and initiate lipid peroxidation (Pyror, 1993) leading to cell death, membrane lysis and subsequent degradation to form lipid peroxide radicals. Biochemically, membrane damage due to reactive oxygen species causes loss of cell structural architecture by disturbing the fluidity and permeability. This affects the influx and aflux of the cellular contents causing cellular swelling, and lysosomal damage by releasing hydrolytic enzymes which lyse the cell and results in serious damage (Wefers and Sies 1983). Autoxidation of cellular components like thiols, H₂O₂ by spontaneous enzyme reaction.
1.6 RESEARCH OBJECTIVES

The present study aimed to investigate the phytochemical, biochemical and pharmacological activity of leaf extract of *Melia Azadirachta* and flower extract of *Borrassus Fiabellifer* and the active principles. The present study is carried out with the following objectives.

1. To select plants and to collect leaves and flowers of identified herbal plants to carry out the study.

2. To investigate preliminary phytochemical constituents present in different extracts of the above two herbs and identification of flavonoids and alkaloids from samples of *Melia Azadirachta* and *Borrassus Fiabellifer* by GC-MS analysis.

3. To examine the nephroprotective effect of extracts of *Melia Azadirachta* and *Borrassus Fiabellifer* against H$_2$O$_2$ induced nephrotoxicity on in VERO cell lines.

4. To evaluate hepatoprotective activity, Nephroprotective potential of normal and in experimental animal liver and kidney injury induced by Paracetamol - *Invivo* study

5. To evaluate the biochemical parameters, such as serum urea, uric acid and creatinine using colorimetric procedure.

6. To estimate the effect of treatment with ethanolic extract of *Melia Azadirachta* and *Borrassus Fiabellifer* on the blood hematological parameters such as, MCH, MCHC, Gran, PLC, MCV and PCV.
7. To determine nephroprotective potential of the two herbal extracts through the antioxidants such as GSH, SOD, GPx, CAT and TBARS

8. To observe and visualize the Histopathology of kidney tissue before and after the treatment of the two herbal extracts.

1.7 ORGANIZATION OF THESIS

Chapter 1 introduces with overall structure and functionalities of liver and kidney. This chapter also gives account on drug induced hepatotoxicity and nephrotoxicity.

Chapter 2 presents the medicinal properties of the two selected herbs *Melia Azadirachta* and *Borrassus Flabellifer*.

Chapter 3 elaborates about GC-MS study to identify the phytoconstituents, along with the preparation of ethanolic extracts of the two herbs. This chapter incorporates the division of animals into several groups to conduct the experiments through *invivo* study. This chapter provides the detailed procedures used to examine the biochemical parameters, hematological parameters and antioxidants required for this study. Chapter 3 also includes the procedure to perform histopathological study.

Chapter 4 presents the results and discussion on screening of photochemical constituents using GC-MS.

Chapter 5 discusses the results obtained for the *invitro* study of VERO cell lines for the nephroprotective nature of ethanolic extracts of MA.
Chapter 6 provides the results of *in vivo* study, biochemical parameters, antioxidants, hematological parameters for the nephroprotective nature of ethanolic extracts of MA and BF, and the histopathological observation.

Chapter 7 summarizes the work carried out in this dissertation along with the research contributions and provides a lead to future work.