1. INTRODUCTION

Man has been using plants for medicinal purposes from time immemorial. It may be difficult to ascertain how and where this aspect of man-plant relationship started. On the basis of empirical experience through generations, human societies developed a certain system of herbal medicines.

The herbal wealth of India and knowledge of their medicinal properties have a long tradition, as referred to in Rig Veda and other ancient literature. The topography of India which is in the tropical belt with its varied climatic zones makes it a vast storehouse of medicinal plants. Indigenous people all over the country have an instinctive knowledge of the therapeutic uses of the plants of their environment. The tribal bodies of India and Africa are a treasure house of knowledge of traditional herbal medicines.

Plants have served mankind since time immemorial as they are important components of medicines and help to alleviate chronic diseases. The near past was considered the synthetic era, as pharmaceutical industries were producing wide variety of synthetic drugs. With passage of time, frequent use of synthetic drugs caused severe side effects, and many microbes became resistant against these synthetic drugs. On the other hand synthetic drugs are expensive and large populations cannot get benefit from these drugs.

During the last decades, a global trend is again turned towards naturopathy and now days green pharmaceuticals are matter of high concern due to minimum side effects, and being cost effective. The importance of herbal medicines in India is much high as 75% population live in rural areas and
traditional healers are their first point of contact. Herbal medicines are getting more attention in big cities also. Alternative ways of treatment having safe and less disruptive are the first choice among both rural and urban population.

It is need of time to conduct maximum scientific research in the field of herbal medicine to exploit the indigenous resources to solve the health problem of people of India. Medicinal plants have an appreciable role in the development of modern medicines as many diseases like cancer, hepatic diseases and arthritis have no complete cure in allopathy. The bioactive compounds of medicinal plants are used as chemotherapeutic agents where no satisfactory cure is present in modern medicines.

Natural products including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The currently accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies.

The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as the important source of future medicine and therapeutics. The future of natural products drug discovery from natural products will be more holistic, personalized and will involve the wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can accrue to the patients and the community.
1.1. LIVER

An adult human liver normally weighs between 1.4-1.6 kg (3.1 - 3.5 lb) (Cotran Ramzi et al., 2005) and is a soft, pinkish-brown, triangular organ. It is the largest internal organ and the largest gland in the human body. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm. The liver lies to the right of the stomach and overlies the gallbladder.

1.1.1. Blood Flow

The liver receives a dual blood supply consisting of the hepatic portal vein and hepatic arteries supplying approximately 75% of the liver's blood supply. The hepatic portal vein carries venous blood drained from the spleen, gastrointestinal tract and its associated organs. The hepatic arteries supply
arterial blood to the liver, accounting for the remainder of its blood flow. Oxygen is provided from both sources; approximately half of the liver’s oxygen demand is met by the hepatic portal vein, and the other half is met by the hepatic arteries (Benjamin et al., 2008). Blood flows through the sinusoids and empties into the central vein of each lobule. The central veins coalesce into hepatic veins, which leave the liver and empty into the inferior vena cava.

1.1.2. Functions of 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 is 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 B₁₂.

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.

1.2. LIVER INJURY

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 (Vinay Kumar et al., 1997). Liver injuries may be viral or caused by drugs, chemicals and alcohol.

1.2.1. 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 Brechet, 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; Pawlobky, 2004; Vandalii et al., 2004). Hepatitis-C will become an increasingly important cause of morbidity and mortality for HIV infected persons (David Macdougall, 2000).
1.2.2. Drug Induced Liver Injury

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).

1.2.3. Paracetamol (Acetaminophen) induced toxicity

Paracetamol (Acetaminophen), acetyl-para-aminophenol (APAP), a p-aminophenol derivative is an active metabolite of both acetonilide 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; Ypar 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).
1.2.4. 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 (Bethesda, 1998). 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 10000 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.

1.2.5. 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.

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.

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.

1.2.6. 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 Ca\(^{2+}\) homeostasis, with increase of the cytosolic 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, Appotosis inducing factor (AIF), and endonuclease G from the mitochondrial intermembrane space.
Reactive oxygen species and peroxynitrite induce the mitochondrial membrane permeability transition (MPT), which causes the collapse of the mitochondrial membrane potential, eliminates ATP synthesis, and causes further release of mitochondrial proteins. 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$^{2+}$/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 in vivo 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.

1.3. LIPID PEROXIDATION

The administration of APAP to rats exerts hepatotoxic effects through lipid per oxidation. Lipid per oxidation 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).

1.3.1. **Oxidative Stress**

Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid per oxidation 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).

1.3.2. 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 peroxy radical (Malland et al., 1983; Eslarbaker et al., 1991). The peroxy 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 Raji, 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).

1.3.3. Antioxidants

“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). 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.

1.3.4. 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 H$_2$O$_2$ and catalase that breaks it down to water (Sies, 1996; 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, 1996; Cadenas and Packer, 1996).

1.4. KIDNEY

The kidneys are paired organs with several functions. They are seen in many types of animals, including vertebrates and some invertebrates. They are an essential part of the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure. In producing urine, the kidneys excrete wastes such as urea and ammonia; the kidneys also are responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, renin, and erythropoietin. Located behind the abdominal cavity in the retroperitoneum, the kidneys receive blood from the paired renal arteries, and drain into the paired renal veins.

Each kidney excretes urine into a ureter, itself a paired structure that empties into the urinary bladder. Renal physiology is the study of kidney function, while nephrology is the medical specialty concerned with kidney diseases. Diseases of the kidney are diverse, but individuals with kidney disease frequently display characteristic clinical features. Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes, renal cysts,
acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction.

1.4.1. Blood Supply

The kidneys receive blood from the renal arteries, left and right, which branch directly from the abdominal aorta. Despite their relatively small size, the kidneys receive approximately 20% of the cardiac output. The interlobar arteries then supply blood to the arcuate arteries that run through the boundary of the cortex and the medulla. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply the glomeruli. After filtration occurs, the blood moves through a small network of venules that converge into interlobular veins. As with the arteriole distribution the veins follow the same pattern, the interlobular provide blood to the arcuate veins and then back to the interlobar veins which come to form the renal vein exiting the kidney for transfusion for blood.
1.4.2. Functions of kidney

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and regulation of blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions; these include renin, angiotensin II, aldosterone, ant diuretic hormone, and atrial natriuretic peptide, among others.

Many of the kidney's functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron. Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are filtered from the blood to make an ultra-filtrate that will eventually become urine. The kidney generates 180 liters of filtrate a day, while reabsorbing a large percentage, allowing for only the generation of approximately 2 liters of urine. Reabsorption is the transport of molecules from this ultra-filtrate and into the blood. Secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine.

The kidneys excrete a variety of waste products produced by metabolism. These include the nitrogenous wastes urea from protein catabolism, and uric acid, from nucleic acid metabolism. Two organ systems, the kidneys and lungs, maintain acid-base homeostasis, which is the maintenance of pH around a relatively stable value. The kidneys contribute to acid-base homeostasis by regulating bicarbonate (HCO$_3^-$) concentration.
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 acetaminophen 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 Ndeacetylase 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.

1.6. NATURAL PRODUCTS AND MODERN THERAPY

Natural products from plants are a rich source of antioxidants used to cure various ailments. The study of naturally occurring compounds obtained from plants has progressed to its present state of development after a long time by passing through different stages. The world of nature abounds in medicinal compounds, which constitutes a fascinating and fruitful area of scientific investigation.

Health and well-being has been a subject of man's primary concern since time immemorial. From his early experiments with herbs and plants growing in his environment and treatment of disease, man was eventually able to establish empirical systems of medicine. The use of plants, plant extracts or pure compounds isolated from natural products to treat diseases is a therapeutic modality, which has stood the test of time even if much of the science behind such therapy is still in its infancy. Phytochemical examination of plants and animal cure available to earlier civilizations has often shown that trees contained active principles, responsible for therapeutic success.

Presently, synthetic drugs outnumber those of natural origin in modern medicine, and research into the isolation and pharmacology of natural products now lags far behind that of synthetic drugs. Nevertheless, there are still many drugs which have their origin in natural products derived from animal or
vegetable sources. Indeed today many, if not most, pharmacological classes of drugs include a natural product prototype. Morphine, digoxin, quinine, atropine, reserpine, physostigmine, pilocarpine, vinblastine, vincristine, artemisinin and taxol are a few examples of what medicinal plants have given us.

There has been a resurgence of scientific interest in medicinal plants during the past 20 years, being rekindled by the world-wide importance of medicinal plants and crude drugs in traditional medicine. Moreover, empirical studies on medicinal plants revealed the fact that for a plant extract to be active clinically, it is not necessary for the active component to be isolated and the structure established.

A large number of crude plant extracts are now being utilized in naturopathic remedies in addition to the purified natural substances. Although a large proportion of the human population uses herbal remedies, only a limited number of plants have been investigated pharmacologically. The inherent biological complexity in plants makes it imperative to evaluate their safety, efficacy and quality during development of plant-based drugs.

In India, the earliest record of the medicinal plants comes from the oldest exposition of human knowledge. The method of treatment without adverse effects or cumulative toxic effects is a main drawback of some chemical compounds of modern medicine. Therefore, medicinal and aromatic plants growing wild in forests are being utilized since time immemorial. It is often ethno medical or ethno pharmacological experience or knowledge that leads to the testing a particular object for its usefulness.
The demonstration of the presence of natural products, viz. polyphenols, alkaloids, flavonoids and other secondary metabolites in medicinal plants will provide a scientific validation for the popular use of them and serve as a guide which may help in the selection of the plants with therapeutic effect. Among the different biomolecules, plant phenolics are one of the target molecules due to their widespread presence and use. It has been shown that various phenolic antioxidants such as falvonoids, tannins, coumarins, xanthones and more recently procyanidins scavenge radicals dose dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies (Czinner et al., 2001).

Flavonoids and spice principles can serve as chemopreventive agents ameliorating the toxicity caused by certain drugs and environmental chemicals or in disease states involving oxidative status. Many of the plants containing polyphenols and tannins which are also being prescribed in Ayurveda (an ancient Indian medical science) and by Hakeems as medicines (Lee, 2004) can promote health and alleviate illness. So, a detailed study of the healing power of plants and a return to natural remedies is an absolute need of our time.

The use of traditional medicine is widespread, and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs (Perry et al., 1999). Most of the phytoconstituents like phenolic compounds (Gonzalez Mejia et al., 2004), saponins, glycosides, flavonoids, steroids and alkaloids are capable of inhibiting hepato and renal cellular damage induced by toxins in both in vivo and in vitro studies (Hasrat et al., 2004).
1.7. HEPATOPROTECTIVE MEDICINAL PLANTS

Liver is an important organ actively involved in metabolic functions and is a frequent target of a number of toxicants (Meyer and Kulkarni, 2001). Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation and have been shown to augment fibrosis as seen from increased collagen synthesis (Geesin et al., 1990). Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues (Thresiamma and Kuttan, 1996).

Free radicals may also be a contributory factor in a progressive decline in the function of the immune system (Pike and Kuttan, 1995). Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes. The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Conventional synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects. Hence there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments.

The majority of ayurvedic herbs such as Cassia occidentalis (Krithikar and Basu, 1999), Scutellaria radix (Hwang et al., 2005), Phyllanthus niruri (Lin et al., 2003), Amaranthus spinosus Linn (Zeashan et al., 2008) and Silybum marianum (Lucena et al., 2002; Das and Vasudevan, 2006; Pradhan and Girish, 2006) are reported to work on multiple biochemical pathways capable of influencing several organ systems simultaneously. However systematic studies
on the indigenous medicinal plants that have been used in the treatment of hepatotoxicity are scanty.

1.8. NEPHRO PROTECTIVE MEDICINAL PLANTS

Human beings are exposed to environmental, occupational and xenobiotics challenges due to modern life style. Enormous free radicals are generated during the exposure to such stressful challenges. In addition the process of metabolism and excretion of xenobiotics may also generate free radicals. These free radicals bind covalently with the tissue macromolecules leading to the cell necrosis (Vijay et al., 2000). Therefore antioxidants are normally administered to treat or prevent the organ toxicities. The herbs containing antioxidant principles are reported to be highly effective in preventing or curing the nephrotoxicites due to the above mentioned challenges.

Nephrotoxicity is of critical concern when selecting new drug candidates during the early stage of drug development (Uehara et al., 2007). Because of its unique metabolism, the kidney is an important target of the toxicity of drugs, xenobiotics, and oxidative stress. In addition, reactive oxygen species derived from chemicals or drugs that are exposed to renal cells appear to mediate renal necrosis, although the mechanisms of free radical toxicity are not well understood. Therefore, it is important to understand the role played by antioxidants during drug-mediated toxicity to determine if they can reduce the oxidative stress induced by reactive intermediates produced by various chemicals and drugs (Sohn et al., 2007; Wu et al., 2007).
Currently, studies are being conducted worldwide to identify protective molecules that can protect the kidney and other organs with few or no side effects. A large number of herbs have traditionally been used to treat drug- or toxin-induced renal diseases, such as *L. divaricata*, *S. simplex*, *P. ginseng*, *A. tataricus*, *C. aurantium*, *S. officianlis*, *A. consanguineum*, and *P. aviculare* (Sohn et al., 2009). In addition, herbal products are often exempt from rigorous governmental regulations, and prescriptions are usually not required for these inexpensive products.

1.9. **FOLKLORE MEDICINAL PROPERTIES OF THE SELECTED PLANTS**

1.9.1. *Caesalpinia sappan* (CS) (Family: *Caesalpiniacea*)

**Taxonomy**
Current name: *Caesalpinia sappan*
Authority: L.
Family: *Caesalpiniacea*

**Synonym(s)**
Biancaea sappan (L.) Todaro

**Common names**
(Tamil): Cemmaram
(Hindi): vakam, vakum
(Burmese): teing-nyet
(English): false sandalwood, Indian brazilwood, Indian redwood, sappanwood
(Filipino): sapang, sibukao
(French): sapang
(Indonesian): kayu sekang, secang, soga jawa
(Lao (Sino-Tibetan)): faang, fang deeng
(Malay): sepang
(Thai): faang, fang som, ngaai
(Vietnamese): tô môc, Vang nhuôm
*Caesalpinia sappan* is a small to medium-sized, shrubby tree, 4-8(-10) m tall; trunk up to 14 cm in diameter; bark with distinct ridges and many prickles, greyish brown; young twigs and buds hairy, brownish. Leaves stipulate, bipinnate, alternate, 20-45(-50) cm long, 10-20 cm broad, with 8-16 pairs of up to 20 cm long pinnae; pinnae with prickles at the base and with 10-20 pairs of oblong, 10-20 mm x 6-10 mm long, subsessile leaflets, very oblique at base, rounded to emarginated at apex. Flowers in terminal panicles, racemes pubescent, primary pendules 30-40 cm long, the flowering 9-15 cm long, bracts ovate-acuminate, about 6 mm long, flowers fragrant, 2-3 cm long, 5-merous; sepals glabrous, petals pubescent, the superior one smaller; calyx tube 3 mm long; corolla yellow, uppermost lobes cuneate, other obovate, all clawed and gland-punctate; stamens 10, filaments densely tomentose in the lower half; ovary superior, pubescent. Fruit a dehiscent pod, glabrous, thick, flattened, obliquely oblong, prominently beaked, woody, polished-brown, 7-10 cm x 3-4 cm, 2-3(-5) seeded. Seeds ellipsoid, flattened, 18-20 mm x 10-12 mm, brown. The generic name is after A. Caesalpini, 1519-1603, Italian physician and botanist.

1.9.1.1. Folklore medicinal properties

The wood of *Caesalpinia sappan* is used for firewood and its energy value is about 25 000 kJ/kg. Timber: The tree is the source of the commercial redwood or Brazilwood (Sosef MSM, Hong LT, Prawirohatmodjo S, 1998). Sapwood is white, heartwood makes up to 90 % of the total volume, is yellow or deep orange when fresh turning to dark red. The wood is straight grained with a fine to moderately fine texture, fairly heavy (600-780 kg/m³), hard and lustrous. It is difficult to dry and susceptible to warping and collapse, but moderately easy to work; it takes high finish and is tough and resistant to termite attack. It is used for inlaying work, cabinet making, violin bows and for walking sticks (Nagai M, Nagumo S, Lee S, Eguchi and Kawai KI, 1986). The stem produces a gum. The heartwood yields a valuable red crystalline dye, brazilin, used on cotton, silk and wool fabrics. Bakam gives bright red and violet shades, and with garcine
produces a chocolate tint. Bark and pods yield similar dyes, pods contain ca. 40% tannin used for production of light leather goods. Roots give a yellow dye. Leaves contain a pleasant smelling volatile oil. A decoction of the wood is a powerful emmenagogue and, because of its tannic and gallic acids, is an astringent used in mild cases of dysentery and diarrhoea. It is also given internally for certain skin ailments (Burkill HM, 1994). The sappan is given as a tonic to women after confinement and to relieve vomiting of blood. It is one of the ingredients in a mixture prescribed for malaria (Duke JA, 1984). The dried heartwood is widely used in oriental medicine, particularly against inflammation. Seeds serve as a sedative. Other products: Seeds are reported to contain trypsin and chymotrypsin inhibitors. Protosappanin A isolated from C. sappan heartwood has a mild sedative effect. Six 3-benzylchroman derivatives (isoflavonoids) were isolated from Sappan Lignum, the dried heartwood of C. sappan (Miyahara K et al. 1986) Screening showed that the methanolic extract had significant anti-hypercholesteremic activity. Brazilin (7,11b-dihydrobenz[b]inden-1,2-d]pyran-3,6a,9,10(6H)-tetrol), the principle component of C.sappan has been found to exhibit hypoglycaemic properties and to increase glucose metabolism in diabetic rats (Kim YouMe et al. 1995).

1.9.2 Clitoria ternatea L. (CT) (Family: Papilionaceae)

Scientific name

Clitoria ternatea  L.

Synonyms

Clitoria albiflora Mattei
Clitoria bracteata Poir.
Clitoria mearnsii De Wild.
Clitoria tanganicensis Micheli
Clitoria zanzibarensis Vatke

Family/tribe

Family: Papilionaceae .
**Common names**

butterfly-pea (Australia); blue-pea, cordofan-pea, honte (French); blaue Klitorie (German); clitoria-azul (Portuguese); azulejo, conchitis, papito, zapatico de la reina, zapotillo, conchita azul, campanilla, bandera, choroque, lupita, pito de parra, bejuco de conchitas (Spanish); cunha (Brazil).

**Morphological description**

*C. ternatea* is a vigorous, strongly persistent, herbaceous perennial legume; stems fine twining, sparsely pubescent, suberect at base, 0.5-3 m long. Leaves pinnate with 5 or 7 leaflets; petioles 1.5-3 cm long; stipules persistent, narrowly triangular, 1-6 mm long, subulate, prominently 3-nerved; rachis 1-7 cm long; stipels filiform, to 2 mm long; leaflets elliptic, ovate or nearly orbicular, 1.5-5 cm long, 0.3-3 cm wide, with apex acute or rounded, often notched, and base cuneate or rounded, both surfaces sparsely appressed pubescent. Flowers axillary, single or paired; colour ranges from white, mauve, light blue to dark blue; pedicles 4-9 mm long, twisted through 180º so that the standard is inverted. Bracteoles persistent, broadly ovate or rounded, 4-12 mm long. Calyx 1.7-2.2 cm long with a few fine hairs; tube campanulate, 0.8-1.2 cm long; lobes triangular or oblong, 0.7-1 cm long, acute or acuminate. Standard obovate, funnel-shaped, 2-5.5 cm long, 2-4 cm wide, notched or rounded at apex, blue with a pale yellow base, or entirely white, a few fine hairs at apex. Pods linear-oblong, flattened, 4-13 cm long, 0.8-1.2 cm wide, with margins thickened, and style persistent, sparsely pubescent when mature, pale brown, dehiscent when dry. Seeds 8-11/pod, oblong, somewhat flattened, 4.5-7 mm long, 3-4 mm wide, olive brown to almost black, shiny, often mottled, minutely pitted: 23,000 seeds/kg. Morphology can vary with different growing conditions. Cv. Milgarra, which has no significant distinguishing morphological characters, is normally towards the upper end of the size ranges of descriptions in the taxonomic literature.

**Distribution**

Native to:


**Indian Ocean:** Mauritius.

Introduced to:

Now widespread throughout humid and sub-humid lowlands of Asia, the Caribbean, Central and South America, and more recently in semi-arid (600–800 mm) tropical Australia.
Uses/applications

Multiple uses. Originally selected as a cover crop. Widely planted as an ornamental on fencerows. Now used for short and medium-term pastures and as green manure, cover crop and protein bank. Increases soil fertility to improve yields of subsequent crops (maize, sorghum, wheat) when grown as green manure or ley pasture. Also used for cut-and-carry and conserved as hay. Hay suitable for goats in Sudan. Used as a revegetation species on coal mines in central Queensland, Australia.

Ornamental and medicinal uses.

**Clitoria** is a genus of about 70 species of evergreen shrubs in the family Berberidaceae, native to eastern Asia, the Himalaya, North America and Central America. They are closely related to the genus Berberis. Botanists disagree on the acceptability of the genus name **Clitoria**. Clitoria ternatea L. (CT) commonly known as Sangupushpam in Tamil is widely distributed in the Tamil Nadu, India.

1.9.2.1. Folklore medicinal properties

Some species of CT were used for treatment of inflammatory skin diseases such as eczema and psoriasis (Bernstein et al., 2006; Lucia et al., 2007). Pharmacological studies confirmed antitussive, antiinfluenza, and free radical scavenging activities (Lucia et al., 2007). Alkaloids such as berberine, isotetrandrine, obaberine, isoquinoline, bisbenzyliso-quinoline, and oxyberberine were also reported to be present in Clitoria species (Donsky et al., 2007; Lucia et al., 2007). Clitoria ternatea L. (CT) is used in
Indian ayurvedic medicine; oral administration of the extract of its roots is used to treat jaundice and fever (Yoganarasimman, 2000; Vijayan et al., 2004).

Indigenous people all over the country have an instinctive knowledge of the therapeutic uses of the plants of their environment. Today, as conventional medicine pursues a more integrated approach to managing disease, natural products and select herbs that influence liver and kidney functions are being revisited and evaluated for their overall health promoting factors. Some notable herbs and nutrients under investigation are garlic, turmeric, and cayenne and milk thistle. In the present work an attempt has been made to evaluate the efficacy of two folklore medicinal plants namely *Caesalpinia sappan* (CS) and *Clitoria ternatea L.* (CT) against the acetaminophen induced hepatorenal toxicity in male *albino* rats.
The present study aimed to investigate the phytochemical, biochemical and pharmacological activity of *Caesalpinia sappan* and *Clitoria ternatea* Linn leaf extract and the active principles. The present study is carried out with the following objectives.

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

2. To investigate preliminary phytochemical constituents present in different extracts and identification of flavonoids and alkaloids from leaf samples of *Caesalpinia sappan* and *Clitoria ternatea* by GC-MS.

3. To analyse biochemical constituents of plant extracts by qualitative and quantitative techniques.

4. To study the antioxidant, hepato-renal protective activity, cytotoxic and free radical-scavenging effects against Hep G2 and vero cell lines.—*In vitro* study.

5. To evaluate hepatoprotective activity, Nephroprotective potential and antioxidant enzymes of normal and in experimental animal liver and kidney injury induced by Paracetamol - *In vivo* study.