CHAPTER 1

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. It is the important organ that controls the internal conditions in the mammalian body (Gray and Lewis 2000). It involves in almost all the biochemical pathways of growth, immunity, nutrient supply, energy provision and reproduction. So it has surprising role in the maintenance, performance and regulating the homeostasis of the body (Avijeet et al., 2007). Liver lies in the right upper part of the abdominal cavity. It is described as having four lobes. The two most obvious are the large right lobe and the smaller, wedge shaped, left lobe. The lobes of the liver are made of tiny lobules (basic functional unit of liver) just visible to the naked eye. These lobules are hexagonal in outline and are formed by cubical shaped cells, the hepatocytes, arranged in pairs of columns radiating from a central vein (Maton et al., 1993 and Williams et al., 1995).

Between two pairs of columns of cells there are sinusoids (blood vessels with incomplete walls) containing a mixture of blood from the tiny branches of the portal vein and hepatic artery (Guyton, 2001). Their arrangement allows the arterial blood and venous blood (with a high concentration of nutritional materials) to mix and come into close contact with liver cells. Some cells, lining the sinusoids, are hepatic macrophages called as kupffer cells. The hepatic artery carries oxygenated blood. The right and left hepatic ducts carry, bile from the liver to the gall bladder. Hepatocytes are polyhedral with five to twelve sides. Their
nuclei are euchromatic and polyploid. Their cytoplasm displays much granular and agranular endoplasmic reticulum, mitochondria, lysosomes, golgi bodies. They contain characteristic type of cytokeratin filaments (Wilson and Waugh, 1996). Fig 1.1 illustrates the structure of liver.

Fig-1.1 Structure of liver

Physiological functions of liver

The various functions of the liver are carried out by the liver cells or hepatocytes. Currently, there is no artificial organ or device capable of emulating all the functions of the liver. Some functions can be emulated by liver dialysis, an experimental treatment for liver failure. The liver is responsible for up to 500 separate functions, usually in combination with other systems and organs. Hepatocytes are responsible for many functions that are important in normal functioning of human body, and they can be basically divided into three categories- regulation and synthesis, storage and purification, transformation and clearance (Paliwal et al., 2009). It regulates the levels of glucose and cholesterol, conversion of glucose to glycogen in the presence of insulin, and hanging liver...
glycogen to glucose in presence of glucagon, synthesis of most plasma proteins, such as alpha and beta globulin, prothrombin, fibrinogen and very low density lipoproteins, synthesis of vitamin A from carotene, the pro-vitamin found in some plants e.g. carrots, green leaves and other vegetables (Harsh Mohan, 2000).

The liver synthesizes angiotensinogen, a hormone that is responsible for raising the blood pressure when activated by renin, an enzyme that is released when the kidney senses low blood pressure, synthesis of bile salts, which are used in the small intestine for the emulsification and absorption of lipids, synthesize cholesterol and use cholesterol to make bile salts, synthesize the constituents of bile from mixed arterial and venous blood in the sinusoids. These include bile salts and bile pigments (Wilson and Waugh, 1996). When blood glucose is high, the liver converts glucose into glycogen and as triglycerides for storage. The liver is a prime storage site for certain vitamins (A, B12, D, E and K) and minerals (iron and copper) which are released from the liver when needed elsewhere in the body. The liver can detoxify substances such as alcohol; excrete drugs and toxins etc., by the process of metabolism. It can also chemically alter or excrete thyroid hormones and steroid hormones such as estrogen and aldosterone. It deaminates the amino acids so that the amino acids can be used for ATP Production. The stellate reticulo-endothelial cells (kupffer cells) of the liver are responsible for phagocytosis of aged blood cells, white blood cells and acts as a sieve for antigens (Tortora and Derrickson, 2006 and Harsh Mohan, 2000).
Liver diseases

To maintain a healthy liver is a crucial factor for overall health and well-being (Rajib Ahsan et al., 2009). Liver disease is still a worldwide health problem. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury gallstones, etc (Wolf, 1999 and Lee, 2003). Based on their duration of occurrence, they are classified as acute disorder (occurs over a period less than 3 months), sub-acute disorder - lasts for 3 to 6 months. Chronic disorder (lasts for more than 6 months).

Drug induced liver damage

Drug- induced liver injury (DILI) is a major health problem that challenges not only the health care professionals but also the pharmaceutical industry and drug regulatory agencies (Ostapowicz et al., 2002). DILI accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and nearly 13% of idiosyncratic liver injury is triggered by some other drugs.

Because of the significant number of morbidity and mortality associated with DILI, the U.S. food and drug administration (FDA) has banned several drugs from the market (Zimmerman, 1999). They include bromfenac (Hunter et al., 1999), ebrotidine (Anonymous, 1998), roglitazone (Kohlroser et al., 2000), risperidone, trovafloxacin and nefazodone etc., (Thames, 2004 and Lasser et al., 2002). The drug induced liver diseases occurs in several ways. They may be dose
dependent toxicity (over dosage), idiosyncratic toxicity (inherited specific genes that control the chemical transformation of that specific drug, causing accumulation of the drug or products of their metabolites that are injurious to the liver), and drug allergy (elicitation of antibodies against certain drugs by immune system). Some of the drugs are directly injurious to liver, some other are transformed by liver into chemicals that can be injurious to liver. When drugs injure the liver and disrupt its normal function, symptoms and signs of liver diseases develop (Bernal et al., 2002).

The disturbances of metabolism occurring in liver diseases are largely due to the failure of the parenchyma cells to carry out vital functions because of infectious or noxious agents, decreased mass of functioning cells, decreased blood supply, impaired nutrition, reactions of other organs to liver damage (Hawk, 1979). Some of the drugs, their metabolites and various chemicals are toxic to hepatic cells and induce various injuries that may range from cholestasis to cell injury of particular structures or organelles of the liver cells and may even cause cell necrosis. Capacity for regeneration is limited if damage is extensive (Isselbacher and Podolsky, 1994).

Pathogenesis

The pathogenesis and mechanisms of hepatotoxicity are still being explored and include both hepatocellular and extracellular pathways. Covalent binding of the drug to intracellular proteins can cause a decrease in ATP levels, leading to actin disruption. Disassembly of actin fibrils at the surface of the hepatocytes causes blebs and rupture of the membrane (Watanabe and Phillips,
Drugs that affect transport of proteins at the canalicular membrane can interrupt bile flow resulting in cholestasis (Trauner et al., 1998). Covalent binding of a drug to the cytochrome p450 enzyme acts as an immunogen, activating T cells and cytokines and stimulating a multifaceted immune response (Cullen, 2005). Activation of the apoptotic pathways by the tumor necrosis factor-alpha (TNF- \( \alpha \)) may trigger the cascade of intercellular caspases, which results in programmed cell death (Faubion et al., 1999).

Certain drugs that inhibit mitochondrial function by a dual effect on both beta-oxidation energy productions by inhibiting the synthesis of nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), resulting in decreased ATP production (Pessayre et al., 2007). Toxic metabolites excreted in bile may cause epithelial injury of bile duct (Sheikh et al., 1997).

**Risk factors for drug-induced liver injury**

Some drugs appear to have selective racial toxicities. For example, blacks and hispanics may be more susceptible to isoniazid (INH) toxicity (Lemke et al., 2007). The rate of metabolism is under the control of cytochrome p450 enzymes and can vary from individual to individual. Although the reasons are unknown, hepatic drug reactions are more common in females. Female gender is suggested to be a risk factor for developing fulminant liver failure (Andrade et al., 2005). Autoimmune hepatitis triggered by drugs is seen almost exclusively in women (Zimmerman, 1999). Diclofenac hepatotoxicity has been reported more frequently in women with osteoarthritis (Watkins and seef 2006).
Alcoholics are susceptible to drug toxicity because alcohol induces liver injury and cirrhotic changes that alter drug metabolism. Alcohol causes depletion of glutathione (hepatoprotective) stores that make the person more susceptible to toxicity by drugs (Batt and Ferrari, 1995). Chronic alcoholics have the risk of developing liver fibrosis during methotrexate therapy, enhances acetaminophen hepatotoxicity by inducing CYP2E1 along with generation of higher levels of the reactive metabolite and depletion of glutathione stores as well as susceptibility to liver damage from isoniazid, halothane and cocaine (Kaplowitz, 2007).

Apart from accidental exposure, hepatotoxic drug reactions are rare in children. Elderly persons are at increased risk of hepatic injury because of decreased clearance, drug interactions, reduced hepatic blood flow and variation in drug binding. In addition, poor diet, infections and prolonged hospitalizations are important reasons for drug-induced hepatotoxicity. As a whole increasing age appears to be a risk factor for developing DILI and is also a determinant of the appearance of a cholestatic/mixed type of damage (Andrade et al., 2005). Hepatocellular damage was inversely correlated with age. Conversely, valproic acid hepatotoxicity occurs more often during the first 3 years of life (Kaplowitz, 2007).

A unique gene encodes each p450 protein. Genetic differences in the p450 enzymes can result in abnormal reactions to drugs, including idiosyncratic reactions. Persons who are malnourished and persons who are fasting may be susceptible to drug reactions because of low glutathione stores (Batt and Ferrari, 1995). The presence of chronic hepatitis B, hepatitis C infection or co-infection
with HIV increases the risk of isoniazid hepatotoxicity or elevated transaminases (Bonfanti et al., 2001). Obesity, Diabetes mellitus type 2 and insulin resistance are known risk factors for steato-hepatitis and have also been shown along with psoriasis to increase the risk of developing liver fibrosis during methotrexate therapy (Rosenberg et al., 2007).

**Clinical features**

Liver disease caused by drugs and chemicals are characterised by symptoms like fatigue, vague abdominal pain, easy bruising due to decreased production of blood clotting factors by the diseased liver, itching, loss of appetite, yellowing of skin, fluid accumulation in legs (edema), white or clay-colored stools, depression and mental confusion or coma. An overstressed liver can impair detoxification and manifest in what may appear to be unrelated symptoms, such as dyspepsia, headaches, menstrual irregularities, bone pain, and muscle stiffness (Gupta and Singhvi, 2011, Arroyo, 1996, Sushma et al., 1992, Centers for diseases control and prevention, 2012 and Appenrodt et al., 2009).

**Clinical diagnosis**

Biochemical analysis is imperative in the diagnosis of both liver disease and liver failure caused by drugs or chemicals. Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) also respectively reflect hepatocellular and biliary injury. Acute hepatic injury can be recognized by the presence of jaundice or non-specific symptoms of acute illness accompanied by elevation of AST and/or ALT activities (Bergmeyer et al., 1978). Increased activity of the liver specific enzyme gamma glutamyl transferase (GGT) reflects hepatocellular and biliary injury.
Liver function tests are abnormal when approximately 60-70% of liver function is lost and includes increased level of conjugated bilirubin due to the disease of the liver or bile ducts. There may be a decrease in BUN (Blood urea nitrogen) and albumin in chronic conditions (Pascucci and Grisley, 1983). High concentrations of ammonia are seen with deficiency of urea cycle enzymes (Batsshaw, 1994), in Reye’s syndrome (Heubi et al., 1984) and with acute or chronic hepatic encephalopathy (Stahl, 1963 and Butterworth et al., 1987).

Prothrombin time (PT) is reproducibly increased, usually at least 3 sec beyond the population mean, in acute ischemic (Dufour and Teot 1998, Fuchs et al.,1998) and toxic hepatitis (Singer et al., 1995), but is rarely elevated more than three seconds in viral (Willner et al., 1998 and O’Grady et al., 1989) or alcoholic (Mendehall,1981) hepatitis. PT is often elevated in obstructive jaundice. In chronic hepatitis, PT is typically within reference limits, but increases as progression to cirrhosis (Bonacini et al., 1997).

Ultrasound examination and liver biopsy are the two most commonly used ancillary tests for detecting liver disease. Ultrasound exam may reveal dilated bile ducts, biliary sludge, biliary stones, hepatic fibrosis, hepatomegaly, smaller than normal liver, hepatic lipidosis and hepatic masses. Liver biopsy is best performed after the liver has been visualized by ultrasound examination. Liver biopsies are the best method used to determine the amount of fibrosis, inflammation, predominant location of disease (Durham et al., 2003).
Management and treatment of liver damage

The medications include anti-inflammatory, immunomodulatory (Kamath and Kim, 2007 and Worman, 1997), corticosteroid, antiviral drugs (Toniutto et al., 2008), S-adenosyl-L-methionine (Wang et al., 2006), Lipoic acid (Packer et al., 1995), dipeptide caspase inhibitor (Ueno et al., 2009), ursodeoxycholic acid (UDCA), a hydrophilic bile acid with putative immunomodulatory capacities (Miyaguchi and Mori, 2005 and Rolandi et al., 1991), and prednisone in combination with azathioprine is also preferred for treating liver diseases (Ishibashi et al., 2007). The current modern therapeutic strategies are not efficient enough for the complete removal of liver hazard, without provoking adverse drug reactions. While a curative agent has not yet been found in modern medicine, the current usage of corticosteroids and immunosuppressive agents only brings about symptomatic relief (Handa et al., 1986). Furthermore, their usage is associated with risk of relapses and danger of side effects.

As per Hepatitis Foundation International, hepatitis B virus is one of the most serious killers in the world. Interferon-based therapy is a standard treatment in modern medicine for chronic viral hepatitis and its use is associated with the risk of relapse and danger of side effects such as depression and suicide. Conventional medicine does not provide efficient remedies for liver diseases. Ribavirin, corticosteroids, nucleoside analogs and thymosin are the usual additives to this treatment (Davis and Rodriguez, 2001 and Okamoto et al., 2001).

Omega-3 fatty acids (fish oil) have been shown to reduce inflammation, which is a distinctive feature of liver disease and cirrhosis (Barham and Edens,
2000). Since cirrhosis is the result of chronic injury to the liver from free radicals, antioxidant therapy may slow down the progression of the disease. Studies have found that people with cirrhosis have low levels of vitamin C and vitamin E (Prakash and Joshi, 2004). Administration of the branched-chain amino acids (BCAAs) like leucine, isoleucine, and valine after surgery, improves the recovery of liver function in hepatic carcinoma (Meng et al., 1999). Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects. Hence, the ultimate treatment of severe liver damage is surgical liver transplantation. Current medical treatments for these liver diseases are often ineffective, and therefore efforts are being made to seek new effective medications (Ahmed and Khater, 2001). In spite of tremendous strides in modern medicine, there are hardly any any drugs that stimulate liver function, and offer protection to the liver from damage or help regeneration of hepatic cell (Chaterjee, 2000). Therefore, due importance has been given globally to develop plant based hepatoprotective drugs effective against a variety of liver disorders (Mohamad Saleem et al., 2010).

In case of drug induced liver diseases the first step is to discontinue the suspected drug. Rapid improvement after withdrawal of the drug is strong evidence in favour of a toxic etiology of the liver disease. The association is even stronger if the 50% decrease in the levels of liver enzymes occurs in the first 8 days after stopping therapy (Danan and, Benichou 1993). However, Specific therapy against drug-induced liver injury is limited to the use of \(N\)-acetylcysteine in the early phases of acetaminophen toxicity. L-carnitine is potentially valuable in cases of valproate toxicity (Bass, 2003). But it is not possible for everyone to
perform the above preventive measure. Because of this and other limitations the use of herbal remedies is on the rise.

**Traditional system of medicine**

Medicinal plants are nature’s gift to mankind. About 8000 plants are recognized as medicines in alternate systems of medicine such as ayurveda, homeopathy, siddha, folk and unani (Gopalakrishnan *et al.*, 1980 and Chopra *et al.*, 1956). Crude extract of medicinal plants are used in ayurvedic preparations. In India more than 500 medicinal plants species are listed to treat various diseases. Medicinal plants are natural resources yielding valuable phytochemical products which are often used in the treatment of various diseases. India with its biggest repository of medicinal plants in the world may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive compounds in the formulation of pharmaceuticals and cosmetics etc (Tiwari, 2008).

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects (Kamboj, 2000). This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998). Since ancient times of civilization, people have been relying on plants as either prophylactic or therapeutically arsenal to restore and maintain health and plants are well known as an important source of many biologically active compounds (Rabe and Staden, 1997). Natural products derived
from plants sources such as flavonoids, terpenes and alkaloids have received considerable attention due to their diverse pharmacological properties including inflammatory, antipyretic and analgesic activities (Witherup et al., 1990 and Shukla et al., 2010).

Consumption of natural products reduces the risk of developing pathological conditions including cancer, nervous system disorders, cardiovascular, genetic and inflammatory diseases (Newman and Cragg, 2007). Plants are the rich source of active ingredients for health care products with many blockbuster drugs being directly or indirectly derived from plants (Newman et al., 2000). However, many high value plant-derived natural products remain undiscovered or unexplored for their pharmacological activity (Raskin et al., 2002).

Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China (Xia dynasty) and India (Vedic period). The use of medicinal plants in curing diseases is as old as man (Aibinu et al., 2007). The World Health organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments (Kamboj, 2000).

These plants which are found in our environment enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox medicine (Akah and Nwabie, 1994). India has been identified as one of the top twelve mega biodiversity center of the world. This is because India has a vast area with wide variation in climate, soil, altitude, latitude etc., In India thousands of species are
known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Tanaka et al., 2006).

**Herbal home remedies**

Drinking 1 glass of water with the juice of 1 lemon and a pinch of salt is beneficial for treating liver disease. Mixing 1 teaspoon of the juice extracted from the black seeds of the papaya fruit with 10 drops of lemon juice and drinking this twice a day for a month will gives best result. Drinking buttermilk with some salt and roasted cumin seeds is excellent for treating liver disease. Carrot juice (300 ml) mixed with 100 ml of either cucumber juice or spinach juice can be consumed daily for at least a month for improving the health of the liver (Boericke William, 1998).

**Herbal treatment**

In ayurveda the word yakrut means liver (Yak- circulation; rut-action). The liver diseases are named as yakrit vridhhi which shows abnormal accumulation of bile and lack of digestion and poor appetite. According to ayurvedic principle the treatment of liver disease is to re-stimulate the liver to excrete bile without accumulation (Vasanth Lad, 1997). *Silybum marianum, Andrographis Paniculata, Foeniculum vulgare, Ficus carica, Aegle marmelos* were some of the herbs which tone up the liver and digestive system (Anil kumar, 2012).

*Hybanthus enneaspermus* Muell, belonging to the family Violaceae, is a herb or under shrub distributed in the tropical and subtropical regions of the world.
abundant from Bundelkund and Agra to Bengal and Sri Lanka, Tropical Asia, Africa and Australia. It is an herb, often with woody troches, found in the warmer parts of India. The plant is popularly known as *Ratanpurus* (Hindi). In the Ayurvedic classics this plant is known as Sthalakamalam and Padma (Singh, 1988).

Several studies have been carried out to investigate the medicinal properties of *Hybanthus enneaspermus* like antimicrobial activity against pathogens of female reproductive tract (Sahoo *et al.*, 2006), against gram positive and negative bacteria (Retnam, 2003), cardioprotective (Radhika *et al.*, 2011), hepatoprotective and antioxidant effect (Vuda *et al.*, 2012), nephroprotective (Manjunath Setty *et al.*, 2007), anti-diabetic and free radical scavenger (Patel *et al.*, 2011), anti-arthritic (Tripathy *et al.*, 2009), anti-fertility (Nathiya and Senthamil selvi, 2013), anti-allergic and analgesic effect (Tamilmozhi *et al.*, 2013). But to date, no data exists to explain the possible mechanism, phytochemical constituents and the amount of phenolic acids responsible for hepatoprotective and antioxidant effect present in the plant. In this regards the present study was carried out using an experimental model.
About the plant: *Hybanthus enneaspermus* (F.Muell)

**Synonym:** *Ionidium Sufftricosum*

**Fig. 1.2 Photograph** of *Hybanthus enneaspermus*

<table>
<thead>
<tr>
<th>Systemic Position (Bentham and Hooker)</th>
<th>Vernacular names</th>
</tr>
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<tbody>
<tr>
<td>Kingdom: Plantae</td>
<td>English: Spade flower</td>
</tr>
<tr>
<td>Order: Malphighiales</td>
<td>Tamil: Orithalthamarai</td>
</tr>
<tr>
<td>Family: Violaceae</td>
<td>Malayalam: Orilathamatai</td>
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<tr>
<td>Genus: <em>Hybanthus</em></td>
<td>Hindi: RatanPurush</td>
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<td>Species: enneaspermus</td>
<td>Sanskrit: Ratnapurusha</td>
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<td>Kannada: Purusharatna</td>
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Print to PDF without this message by purchasing novaPDF (http://www.novapdf.com/)
*Hybanthus enneaspermus* is placed in the International Plant Name Index (IPNI) with the Identification number 866640-1. Root is diuretic, used in bowel complaints of children and administered as an infusion in gonorrhoea and urinary tract infection (Asima Chatterjee *et al.*, 1992). Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhea, leucorrhoea, dysuria, and sterility (Yoganarasimhan, 2000). The plant is also attributed to its antimicrobial (Rajakaruna *et al.*, 2002) and anti-plasmodial action (Weniger *et al.*, 2004). In some part of India, the plant is used to treat diabetes and is also having anti-oxidant property and free radical scavenging activity (Dass *et al.*, 2004). The plant has been reported to have anti-inflammatory, antitussive and anticonvulsant activity (Nichans and Samuelson, 1968).

**Habitat and morphology**

Branching herbs, leaves - 1-3x0.2-0.3 cms, Sub-sessile, linear – lanceolate, acute at apex, attenuate at base, glabrescent. Flowers- pink, solitary; pedicels 0.7-1cm, long. Calyx – membranous, lobes 5, 2-3, mm. long sub canal, lanceolate ciliate. Corolla- lobes 5, unequal; upper 2-3 mm. long oblong; laterals 3-4 mm. long falcate; lower 0.8- 1x0.6-0.7 cm, orbicular. Stamens 6, connate; filaments short; anterior 2 filaments with filiform appendages, Ovary- unilocular, multi-ovuled; style 2-3 mm. long clavate capsule 3-4 mm across, subglobose (Keshava murthy and Yoganarasiman, 1990).

**Traditional medicinal uses**

*Hybanthus enneaspermus* is traditionally used as a demulcent and tonic by the tribal peoples-Bagatas, Konda Doras and Valmikis in Andhra pradesh district (Reddy *et al.*, 1989). The fruits and leaves are used as antidotes for scorpion stings...
and cobra bites by the Yanadi tribes in Andhra Pradesh (Sudarsanam and Sivaprasad, 1995). Decoction of leaves and tender stalks are demulcent. The fruits are used to treat scorpion sting (Boominathan et al., 2003). It is bitter, acrid, used in urinary-calculi, strangury, vomiting, wandering of the mind, urethral discharges, blood disorders, asthma, epilepsy, cough and it also gives tone to the breasts (Kirtikar and Basu 1988). In Ayurvedic literature, the plant is reported to cure conditions of “Kapha” and “Pitta” (bile), jaundice in India (Gopal and Shah, 1985), and it is anti-malaria (anti-plasmodial) (Patrice Jomnang and Francoise Benoit, 2007). The plant is used by some tribe in Orissa to cure diabetes (Dass et al. 2004).

**Phytochemical chemical constituents**

Plant extract contains alkaloid, aurantiamide acetate, β-sitosterol and isoarborinol (Asolkar et al., 1992). Nectar contains high amount of amino acids including valine, leucine and glutamic acid (Ghani, 2003). Bio-active components of plants generally like phenol, saponins, alkaloids, amino acids and flavanoids, which are possessing biological activities including anti-cancerous, anti-fungal and anti-inflammatory activities (Arul Doss and Kalaichelvan 2012 and Arumugam et al., 2012). Previously reported that the presence of alkaloids, flavanoids, tannins, cardio glycosides, saponins, and terpenoids like compounds in *Hybanthus enneaspermus* play a major role to cure common sickness (Amutha Priya et al., 2011). Due to the lack of experimental evidence on the biochemical role of *Hybanthus enneaspermus* on hepatotoxicity induced by paracetamol, the study was carried out to elucidate the hepato protective and antioxidant activity of *Hybanthus enneaspermus* plant.
AIM AND OBJECTIVE OF THE STUDY

- To perform physico-chemical analysis and extractive value determination in the crude powder of *Hybanthus enneaspermus* plant.
- To study the qualitative and quantitative phytochemical analysis of primary and secondary metabolites present in different extract (aqueous, hydro-ethanolic, hydro-methanolic and petroleum ether) of *Hybanthus enneaspermus* and best extract chosen for further study.
- To study the hepatoprotective effect of the best extract from the whole plant of *Hybanthus enneaspermus* against paracetamol induced liver damage in rats.
- To study the antioxidant effect of the best extract from the whole plant of *Hybanthus enneaspermus* against paracetamol induced oxidative stress in rats.
- To study the phytochemicals present in *Hybanthus enneaspermus* by GC-MS analysis.
- To quantify the flavonoids and phenolic acids for its antioxidant effect present in *Hybanthus enneaspermus* by HPLC analysis.