CHAPTER 1

INTRODUCTION
1.1 Liver

Liver is the largest gland in the body weighing about 1500g in an adult and accounts for approximately 2.5% of total body weight (Singh et al., 2012; Juza and Pauli, 2014). Liver is called as the metabolic “engine-room of the body” (Vishal, 2013). The liver is divided into two lobes, right and left, a large right lobe and a smaller left lobe are separated by falciform ligament. Liver has 50,000-100,000 lobules (Caruthers, 1997). Each lobule consists of a central vein surrounded by tiny cells (Fig. 1) like hepatocytes, endothelial cells, kupffer cells and stellate cells grouped in sheets or bundles (Jacobs et al., 2010; Ho et al., 2013). The liver metabolizes xenobiotics and drugs into toxic intermediates (Bhattacharjee and Sil, 2006).

Liver performs vital role in wide range of functions such as metabolism of nutrients like amino acids, carbohydrates, lipids, minerals, vitamins; it also helps in blood clotting through synthesis and secretion of plasma proteins; eliminates dead red blood cells from blood circulation; eliminates bacteria; detoxifies chemicals, drugs, xenobiotics, helps in digestion and fat metabolism by excretion of bile salts; and excretion of end products of metabolism through urine (Singh et al., 2012). Liver plays role in both metabolism as well as biochemical transformation (Sahu, 2007).

Fig. 1. Structure of Liver

(From Ho et al., 2013)
1.2 Hepatotoxicity

Toxicity mentions to the unsafe impacts of substances on an entire living being, in a creature, bacterium or plant, and in addition the substructure of life forms, for example, a cell (Cytotoxicity), or organ (Organotoxicity) and liver Hepatotoxicity (Bahar et al., 2013).

Hepatotoxicity is most commonly seen in the form of malfunction or damage to the liver due to excess amount of drugs or xenobiotics (Navarro, 2006; Singh et al., 2011). Hepatotoxicants are exogenous substances of clinical relevance which may include an overdose of certain medicinal drugs (acetaminophen, nimesulide, antitubercular drugs like isoniazid, rifampicin etc.), industrial chemicals (alcohol, CCl₄, beta galactosamine, thioacetamide) etc., which causes liver injury (Willett et al., 2004; Bigoniya et al., 2009; Papay et al., 2009; Singh et al., 2011; Pandit et al., 2012). The exact mechanism of drug induced liver injury remains largely unknown, but it appears to involve two pathways – direct hepatotoxicity (Type A or DILI1 (drug induced liver injury1), intrinsic or predictable drug reaction) and indirect hepatotoxicity (Type B or DILI2 (drug induced liver injury2), unpredictable or idiosyncratic drug reaction,) or adverse immune reaction (Bigoniya et al., 2009). The most common direct hepatotoxins are carbon tetrachloride, thioacetamide, acetaminophen, galactosamine, fulvine, phalloidin, ethyl alcohol, aflatoxins etc. Some examples of indirect hepatotoxins are methyl testosterone, chlorpropamide, tetracycline, halothane, phenytoin, methylidopa, sulphonamides, allopurinol, rifampicin etc (Bigoniya et al., 2009). Hepatotoxicity is manifested by different types of injuries, depending on the nature and dose of the chemical. Hepatotoxicity may result into cytotoxic effects (necrosis, apoptosis), cholestasis, steatosis, fibrosis, cirrhosis, hepatitis and liver tumors (Lee, 2003). Hepatotoxicity related symptoms may include jaundice or icterus appearance causing yellowing of the skin, eyes and severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light coloured stools (Chang and Schaino, 2007).
1.3 Prevalence of Liver Disease

Liver diseases are fatal and leading cause of illness and deaths worldwide (Wang et al., 2014a). As per study, liver disorders cause about 18000 to 20000 deaths every year globally (Fatma and Uphadhyay, 2015; Akila and Prasanna, 2014). In United States, about 2-5 % of hospital admissions are due to liver injury out of which 10% results in acute liver failures (Pandit et al., 2012). In United States, rate of liver transplantation is more than 75% for Type B drug reactions (Ostapowicz et al., 2002). The crude incidence of liver disorder is 14 per 100000 per annum globally, whereas the standard incidence is 8.1 per 100000 per annum (Bedi et al., 2016). Acute liver failure rate is up to 13% of the cases in developed nations like USA whereas it is less (5%) in tropical countries like India (MeMahon, 2005).

1.3.1 International Scenario

Acetaminophen or paracetamol is easily available as over the counter drug and has become major reason of self-poisoning in recent years (Ghaffar and Tadvi, 2014). About 50% of self-poisoning cases are due to paracetamol causing 100-200 deaths per annum in UK (Kumar et al., 2005). In USA, 61.8% of paracetamol overdose cases were found unintentional and 30.5% were related with suicidal attempts (Serper et al., 2016). It has been reported as the most common drug overdose either accidentally or unintentionally resulting into acute liver failures (ALF) in the United Kingdom (UK, 60-75% of ALF aetiology), Europe (2% of ALF aetiology in France), Canada, United States (US, approximately 20% of ALF aetiology), and Australia (Ostapowicz and Lee, 2000; Robinson et al., 2000; Ostapowicz et al., 2002; Ayonrinde et al., 2005; Marzilawati et al., 2012). However, the incidence of acetaminophen-induced acute liver failure cases in US has increased exponentially (Larson, 2005). In year 2009, 401 deaths were reported due to acetaminophen overdose by American Association of Poison Control Centers (Sreejith et al., 2015). According to a recent report, about 42% of acute liver failure cases out of more than 80000 emergency visits and 30000 hospitalizations are reported in US (Lancaster et al., 2015).

Alcohol is one of the main causes of end stage liver disease and leading cause of morbidity and mortality worldwide (WHO, 2011; Wang et al., 2014a). Deaths due to alcohol liver diseases are increased since last decade (Mandayam et al., 2004) and
have become a common reason for cirrhosis in Western countries (WHO, 2011). In the USA, second leading cause for liver transplantation is alcoholic cirrhosis (Varma et al., 2010). The mortality rate for alcoholic liver disease (ALD) was 7.9 per 100,000 in the United States (Roizen et al., 1999). In 2006, 22,073 deaths in the United States (excluding accidents/homicides) were related to alcohol, with approximately 13,000 deaths specifically due to ALD (Beier et al., 2011). The Global Status Report on Alcohol and Health by WHO reported highest alcohol consumption in developed world including Western and Eastern Europe (WHO, 2011). European countries reported 1 in 7 male deaths and 1 in 13 female deaths due to ALD in 2004 (WHO, 2012). Nearly 88,000 people (approximately 62,000 men and 26,000 women) die from alcohol-related causes annually, making it the fourth leading preventable cause of death in the United States (CDC, 2013; Stahre et al., 2014). A WHO study in 2012 reported about 3.3 million deaths worldwide, of which 5.9% were caused by alcohol consumption (WHO, 2014). About 3.8% of global mortality is accounted for alcohol consumption (Li et al., 2015).

1.3.2 National Scenario

In India, 33.2% patients were reported with acetaminophen overdose in the study on 1024 patients (Median age 23 years, 82.0% female) from January 2005 to December 2009 (Marzilawati et al., 2012). The data on acetaminophen self-poisoning in India is highly insufficient as compared to that of western countries (Nambiar, 2012).

In India, the prevalence rate of liver injury due to alcohol is higher than that of acetaminophen which is largely attributable to utilization of illegal alcohol (Malik et al., 2015). In India, 5% of all deaths are because of liver diseases for which the most critical culprit is alcohol (Vasudevan, 2011). Alcoholism is the most common cause of fatty liver and cirrhosis in India (Vasudevan, 2011). The prevalence of alcohol consumption ranges from 7% in Gujarat, to 75% in Arunachal Pradesh (Murthy et al., 2010; Punia, 2014). The per capita consumption is 2 litres per adult per year which accounts for 50% of chronic liver diseases (Punia, 2014). In India, mortality rates due to ALD for males (11/100,000) are reported to be higher than that of females (6/100,000) (Nagaraju, 2014). Significantly higher alcohol consumption has been...
recorded among tribal, country and lower socio-economic urban sections (Benegal, 2005; Punia, 2014).

1.4 Risk Factors for Liver Disease

The risk for developing liver disease varies, depending on cause and co-occurrence of other medical conditions. Different risk factors for liver disease (Fig. 2) include age and gender, genetic factors, obesity, arsenic, aflatoxins, dietary supplements, industrial toxins, diabetes, alcoholism, and long-term use of certain medicinal drugs (Acetaminophen) (Lin et al., 2013; Purnak and Yilmaz, 2013; Mehta, 2014; Singh et al., 2016).

Fig. 2. Risk Factors for Liver Diseases

(Figure modified from Singh et al., 2016)

1.4.1 Age and Gender

Hepatic drug reactions are rare in children except accidental exposure. Risk of hepatic injury is higher in adults due to reduced hepatic blood flow, drug-to-drug interactions, variations in drug binding, and lower hepatic volume. Hepatic drug reactions are more common in females, though the reasons are unknown (Mehta, 2014).
1.4.2 Genetic Factors

P-450 protein is in charge of the metabolism of most of the medications. Hereditary varieties in the P-450 compounds can lead to abnormal reactions to drug including peculiar adverse reactions. Variations in the P-450 can be recognized by amplification in polymerase chain reaction of mutant genes. This has prompted the likelihood of future identification of persons who can have anomalous responses to a medication (Mehta, 2014).

1.4.3 Obesity

Risk of liver disease increases with weight and obese individuals are more likely to develop liver complications than non-obese individuals. The fat cells which aggregate in liver of obese persons cause liver damage and scarring (sclerosis) (Lieber, 2003). With increasing weight, the likelihood of liver disease advancement, cirrhosis, and NASH (Non Alcoholic Steato-Hepatitis) goes on increasing (Tolman and Dalpiaz, 2007). Fat accumulation in liver consequently leads to NAFLD (Non Alcoholic Fatty Liver Disease) (Kneeman et al., 2012).

1.4.4 Arsenic

An increased risk in development of some form of liver cancers has been reported due to chronic exposure to naturally occurring arsenic through drinking water (Contaminations in some wells) (Lin et al., 2013).

1.4.5 Aflatoxins

Aflatoxin is a substance made by fungus that contaminates mouldy wheat, corn, soybeans, rice, and some types of nuts which causes cancer. Storage of the food stuff in a moist, warm environment causes this kind of contamination and is more common in warmer and tropical countries (Egner et al., 2001).

1.4.6 Dietary Supplements

Poor nutrition and fasting involves risk of liver disease (Malik et al., 2015). Liver plays key role in regulating the nutritional state and the energy balance in the body. Development of malnutrition is common in patients with hepatic disorders
(Purnak and Yilmaz, 2013). Deficiency of vitamin A and E may aggravate effects of alcohol induced liver damage by preventing regeneration of hepatocytes (Addagudi et al., 2013). This is a particular concern as alcoholics are usually malnourished due to poor diet, anorexia and encephalopathy (Narayanan Menon et al., 2001).

1.4.7 Industrial Toxins

Many chemicals and organic solvents used in different industrial processes may be associated with hepatotoxicity. Industrial toxins include dimethylformamide, trichloroethylene, tetrachloroethylene, xylene, toluene, carbon tetrachloride, and vinyl chloride (Malaguarnera et al., 2012).

1.4.8 Diabetes

Risk of developing chronic liver disease and hepatocellular carcinoma is higher in diabetes patients than in normoglycemic individuals (El-Serag et al., 2004).

1.4.9 Alcoholism

Alcohol consumption is common reason for liver cirrhosis which increases risk of liver cancer (Tome and Lucey, 2004; Galicia-Moreno and Gutierrez-Reyes, 2014). The quantity and duration of alcohol intake increases risk of liver disease (Bruha et al., 2012). One in five heavy drinkers develops alcoholic hepatitis, and one in four develops cirrhosis (Bruha et al., 2012). It is estimated that, individuals consuming more than 200mL alcohol per day for more than 14 years may develop liver disease (Malik et al., 2015). Alcohol also causes increased hepatotoxicity of several xenobiotics (Radhika et al., 2011). Alcohol induced hepatic injury is due to accumulation of reactive oxygen species and consequent lipid peroxidation of cellular layers, proteins and DNA oxidation (Zhou et al., 2002; Galicia-Moreno and Gutierrez-Reyes, 2014).

1.4.10 Long-Term Use of Certain Medicinal Drugs

Long term use of analgesics and antipyretics cause hepatic injury and on prolonged conditions it leads to centrilobular hepatic necrosis (Pandit et al., 2012; Bebnista and Nowak, 2014). Consumption of acetaminophen like drugs may lead to hepatocellular carcinoma (Singh et al., 2012). Oxidative stress plays a central role in
the hepatic damage caused by acetaminophen and antioxidants have been tested as alternative treatment against acetaminophen toxicity (Li et al., 2015). Paracetamol (Acetaminophen) overdose is the most frequent reason for medication induced liver failure in the United States and in Great Britain (Jaeschke and Bajt, 2006). Long-acting medications might bring about more harm than short-acting medications (Mehta, 2014).

1.5 Biochemical Markers

1.5.1 Liver Function Tests

The hepatotoxin causes certain histological changes with typical clinical signs which indicate liver injury (Singh et al., 2011). Clinical assessment of liver damage and injury include assessment of serum biochemical markers like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin, which are known as liver function test markers.

Based on the mechanism of injury, hepatotoxicity can be broadly classified into hepatocellular and cholestatic injury (Lee, 2003; Singh et al., 2011). Increase in SGOT, SGPT, and ALP levels is an indication of cellular leakage. It further causes decreased functional integrity of hepatic cell membranes resulting into hepatocellular damage (Basu et al., 2012). Total bilirubin indicates functional status of the hepatic cells (Basu et al., 2012). The obvious signs of hepatocellular injury primarily involve increase in SGOT, SGPT preceding increase in total bilirubin level and small increase in ALP level (Singh et al., 2011). In cholestatic injury, elevation in ALP level is more prominent as compared to that in SGOT and SGPT. Generally mixed type of injuries, involving both hepatocellular and cholestasis injuries, are observed clinically (Teschke, 2009). The ratio of SGOT: SGPT plays an important role in deciding the type of liver damage by hepatotoxins (Mishra, 2012). SGOT: SGPT ratio in hepatocellular damage is greater than or equal to five while it is less than or equal to two during cholestatic liver damage (Singh et al., 2011). The ratio ranges between two and five for mixed type of liver damage (Singh et al., 2011).
1.5.2 Total Protein

The liver is the major source of most of the serum proteins and amount of serum total proteins indicate the functional status of the hepatic cells (Gupta et al., 2007; Thapa and Walia, 2007; Basu et al., 2012). Liver cells synthesize albumin, fibrinogen, prothrombin, alpha-1-antitrypsin, hepatoglobin, ceruloplasmin, transferrin, alpha foetoproteins etc., while damaged liver shows decreased levels of these plasma proteins (Thapa and Walia, 2007; Mishra, 2012).

1.5.3 Lipid Profile

The liver is a major organ regulating lipid metabolism and it synthesizes and metabolizes cholesterol, bile acids and phospholipids (Werner et al., 2000). Liver synthesizes nearly 80% of the cholesterol produced in the body from Acetyl-CoA via a pathway that connects metabolism of carbohydrates with that of lipid. Liver can synthesize, store and export triglycerides (Dean et al., 2009; Rui, 2014).

Acetaminophen intoxicated rats show elevated levels of cholesterol and triglycerides, indicating hepatic damage and consequent impairment in fat metabolism (Haldar et al., 2011). The liver controls cholesterol and triglyceride levels in the body by assembling, secreting, and taking up various lipoprotein particles (Cox and Garcia-Palmieri, 1990). During these functions, loss of lipid and protein components causes change in structure of VLDL particles (Marais, 2004). The resulting LDL particles are then returned to the liver through LDL receptors on hepatocytes (Marais, 2004). Greater increase of LDL and VLDL may also cause a greater decrease of HDL, disturbing lipid metabolism in liver (Al-Assaf, 2013).

1.5.4 Oxidative Stress Markers

Oxidative stress is one of the important reasons for pathogenesis of hepatic dysfunction in humans (Rahman et al., 2012) and animals (Abd Ellah et al., 2009; Abd Ellah, 2010). Toxicity of a xenobiotic is also affected by the generation of reactive oxygen species (ROS) by a few different mechanisms including mitochondrial damage, activation of cytochrome P450 2E1 (CYP2E1), and infiltration of Kupffer cells and granulocytes (Arteel, 2003; Smathers, 2006; Albano, 2008). Oxidative stress within the cells by partially reduced free oxygen species such as, \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \) and \( \text{OH}^- \) Causes damage to hepatic parenchymal cells leading to hepatic injury.
Liver releases free radicals during detoxification of chemicals, drugs and other toxic materials (Abd Ellah, 2011). The elevation in free radicals and decreased scavenging potential of the cells is observed in hepatic injury (Jothy et al., 2012). Liver cells have endogenous antioxidant system comprising of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA) which offers protection against oxidative stress (Haldar et al., 2011). Elevated levels of antioxidant enzymes and decrease in MDA helps in hepatoprotection while decrease in antioxidant enzyme activities and increased MDA results in hepatocellular damage which leads to degradation of cellular macromolecules in liver by chemical induced toxicity (Janero, 1990; Durairaj et al., 2007; Palanivel et al., 2008; Haldar et al., 2011).

1.6 Management of Hepatic Diseases

In today’s world, liver diseases are a major serious health problem. Despite considerable progress in modern medicine, the drugs or agents which can stimulate liver function or help regeneration of hepatic cells or offer protection to the liver damage are still wanted (Vishal, 2013). In addition, the synthetic drugs used in modern medicine have been reported to have many undesirable side effects. Hence, there is a recent renewal of interest in search for the natural resources like medicinal plants which have promising potential to offer several herbal medicines with less side effects (Sudaroli, 2013). Thousands of medicinal plants are used worldwide to prevent as well as cure many diseases, but the mode of their protective and curative actions still remains unclear (Pan et al., 2014).

The therapies available to treat liver diseases include surgical procedures, hepatoprotective agents, herbal formulations, medicinal plants, nutritional supplements etc.

1.6.1 Surgical Procedures

Liver transplantation has become an acceptable means with excellent long-term outcomes to treat end-stage liver diseases, but it is very costly. Liver failure due to viral hepatitis (especially hepatitis B and C) is a common indication for liver transplantation (Terrault et al., 2005).
1.6.2 Hepatoprotective Agents

Hepatoprotective agents have recently been given attention due to their roles in the additional treatment of liver disease (Flatland, 2003; Sartor and Trepanier, 2003; Twedt, 2004). These products include both prescription drugs and nutraceuticals. In order for a compound to be used as a drug, it must be harmless and effective for its intended use. The drug can be released in the market only after undergoing an extensive Food and Drugs Administration’s (FDA) drug approval process which is lengthy and costly. Apart from modern drugs, there are several hepatoprotective agents like L-carnitine (Yapar et al., 2007), Vitamin C (Adikwu and Deo, 2013), N-acetylcysteine (Maheshwari et al., 2014), and Milk Thistle (Silymarin) (Vargas-Mendoza et al., 2014). Silymarin is a unique flavonoid complex derived from milk thistle and is commonly used for regeneration of liver cells, decongestion of liver, complementary treatment to the patients of liver cirrhosis and viral hepatitis etc. It is also used for hepatoprotection against industrial chemicals and pharmaceuticals (Das et al., 2011).

1.6.2.1 Silymarin

Silymarin is a standardized extract from the seeds of a plant called milk thistle (*Silybum marianum* L.; Family: Asteraceae). In rural areas, it has been used as a natural remedy to treat liver diseases (Saller et al., 2001). Silymarin helps to protect and enhance the regeneration of liver cells in most of the liver diseases like cirrhosis, hepatitis and jaundice (Flora et al., 1998). Silymarin possess membrane stabilizing, anti-oxidative, anti-lipid peroxidative (Pascual et al., 1993), anti-fibrotic (Jia et al., 2001), and immune-modulatory properties and helps in liver regeneration (Pradhan and Girish, 2006). Studies on human beings demonstrated that about 20-40% silymarin is excreted as sulphates and glucuronide conjugates in bile (Saller et al., 2001). There are a few reports of low level of silymarin toxicity causing allergic skin rashes and gastrointestinal disturbances (Saller et al., 2001).

1.6.3 Herbal Formulations

Numerous medicinal plants and their formulations are used to treat liver disorders in ethno medicine practice as well as traditional system of medicine in India. There are about 600 commercial herbal formulations available in market all over the
world, which are claimed to have hepatoprotective activity (Bedi et al., 2016). In India, about 40 anti-hepatotoxic, patented, polyherbal formulations representing a variety of combination of 93 medicinal plants from 44 families are available (Sharma et al., 1991). More than 700 mono and poly-herbal hepatoprotective preparations from more than 100 plants are in clinical use in the form of decoction, tincture, tablets and capsules. Recent global increase in the utilization of herbal drugs has also been reported in the literature (Girish et al., 2009).

1.6.4 Medicinal Plants

Eastern countries have been using herbal drugs to treat liver diseases since ancient time (Rajaratnam et al., 2014). The ancient Chinese and Egyptian written records are available which describe medicinal uses of plants (Rajaratnam et al., 2014). In ancient India (Vedic period) and China (Xia dynasty), records on use of herbal medicines track back to 2100 BC. The first written reports date back to 600 B.C. with the Charka Samhita of India and the early notes of the Eastern Zhou dynasty of China around 400 B.C (Onyije and Avwioro, 2012).

Ayurveda, an indigenous system of medicine in India, has a long tradition of treating liver disorders with plant drugs. Minimizing side effects and increasing therapeutic efficacy of medicines is the basic need of today. Alternative system of medicine like Ayurveda, Unani etc. has been proved to be effective with minimum side effects. With rich diversity of plants, over 45,000 diverse plant species are found in India out of which about 15,000-20,000 plants have medicinal and therapeutic properties. Of these, only about 7,000-7,500 are being used by traditional practitioners (Bedi et al., 2016). As per WHO report, around three quarters of the world’s population uses herbs and other traditional medicines to cure various diseases, including liver disorders (Chaudhury and Refei, 2001). The medicinal plant such as Guduchi (Sharma and Pandey, 2010), Elephantopus scaber (Ho et al., 2012), Aquilegia vulgaris (Adamska et al., 2003), Strychnos potatorum (Sanmugapriya & Venkataraman, 2006), Tridax procumbens (Ravikumar et al., 2006), Picrorhiza kurroa (Mohd et al., 2012), Silybum marianum (Hermenean et al., 2015), Andrographis paniculata (Nasir et al., 2013), Azadirachta indica (Johnson et al., 2015) and Glycyrrhiza glabra (Sharma and Agrawal, 2014) has proven hepatoprotective properties and are used to treat liver disorders. Guduchi (Tinospora

12
sp.) is one of the most versatile rejuvenating shrubs, also known as ‘Giloya’ in Indian vernacular, and is reported to have many therapeutic applications (Pandey et al., 2012). Guduchi, as it is most commonly called, has been described as “one which protects the body” (Gawhare, 2013).

1.6.4.1 *Tinospora* forms

*Tinospora* (Guduchi) is one of the most commonly used plants for preparation of hepatoprotective ayurvedic formulations. *Tinospora* belongs to family Menispermaceae. *Tinospora* is a climbing or twining shrub (Choudhary et al., 2013; Tripathi et al., 2015) and is found mostly in tropical and subtropical areas of India with different names (Nidhi et al., 2013). More than 32 species of Guduchi are found all over the world (Choudhary et al., 2013). Four different species of *Tinospora* occur in India viz. *Tinospora cordifolia* (Wild.) Miers ex Hook. F. & Thoms, *Tinospora sinensis* (Lour.) Merr., *Tinospora crispa* (L.) Miers ex Hook. f. & Thoms and *Tinospora glabra* (Burm f.) Merrill (Pramanik and Gangopadhyay, 1993). Other common names for Guduchi are Gilo (Arabic), Amarlata (Assamese), Gadancha, Guluncha, Giloe (Bengali), K’uan chu Hsing (Chinese), Culancha (French), *Tinospora* (English), Gado, Golo, Gulo (Gujerati), Giloe, Gulbel, Gurcha (Hindi), Amrytu, Sittamrytu (Malayalam), Ambarvel, Giroli, Gulvel (Marathi), Garjo (Nepali), Gulancha (Oriya), Gulbel (Persian), Gilo (Punjabi, Kashmiri), Amrita, Guduchi, (Sanskrit), Gurjo (Sikkikim), Amridavalli, Niraidarudian (Tamil), Guduchi, Iruluchi (Telugu) and Guruch (Urdu) (Choudhary et al., 2013; Tripathi et al., 2013). In this study we have selected three difference forms of guduchi (Fig. 3):

(a) *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms.
(b) *Tinospora sinensis* (Lour.) Merrill.
(c) Neem-giloe (*Tinospora cordifolia* plant growing on *Azadirachta indica* (Neem tree).
(a) *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms

### Table 1. Classification of *T. cordifolia*

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<td><strong>Botanical name</strong></td>
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**Distribution**

**Global:** India, Sri Lanka, Bangladesh, China Malaysia, Indonesia, Pakistan and Thailand (Raghu et al., 2006). **National:** Plant is distributed throughout the tropical region of India up to 800-1200 m above sea level, extending from Himalayas down to the southern part of peninsular India (Geetha et al., 2007; Nidhi et al., 2013).

**Vernacular Names**

*Guduchi, Madhuparni, Amrita, Amritavallari, Chhinna, Chhinmaruha, Chhinnodhava, Vatasadani, Tantra, Kundalini, Chakralakshanika, Somavalli, Dhira, Vishalya, Rasayani, Chandrahasa, Vayastha, Mandali, Devanirmita* (Sharma et al., 2005).

**Medicinal Properties**

*Tinospora cordifolia* contains alkaloids, glycosides, diterpenoid lactones, sesquiterpenoids, steroids, phenolics, aliphatic compounds and polysaccharides and are rich in protein, calcium and phosphorus (Singh et al., 2013). *T. cordifolia* has been used in Ayurvedic Rasayana due to its immune-modulatory and hepatoprotective activity (Bishayi et al., 2002; Upadhyay et al., 2010). *T. cordifolia* is one of the major constituents of several Ayurvedic preparations and is used preferably for general
debility, dyspepsia, fever, urinary diseases, jaundice, skin diseases, diabetes, anaemia, cancer, liver disorder, heart disease, Parkinson’s disease, emaciations, and hepatitis B and E (Sinha et al., 2004; Ganguly and Prasad, 2011).

(b) *Tinospora sinensis* (Lour.) Merrill

Table 2. Classification of *T. sinensis*

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Distribution

Global: India, Sri Lanka, Nepal, Bangladesh, Myanmar, China, Thailand, Vietnam and Cambodia (Ravikumar and Ved, 2000). National: Plant is distributed throughout the subtropical evergreen or mixed deciduous forests, scrub jungles and forests, on sandy loam, hedges and occasionally in rocky valleys, up to 800 m (eFlora of India, 2014). In India, it occurs in Assam, Bihar, Orissa, Maharashtra, Andhra Pradesh, Karnataka, Kerala and Tamilnadu (Ravikumar and Ved, 2000).

Vernacular Names

Medicinal Properties

*T. sinensis* contains starch and traces of berberin (Jain et al., 2010). *T. sinensis* has been used to treat piles, ulcerated wounds, liver complaints, chronic rheumatism, tuberculosis, debility (weakness), burning sensations during urination, ear pain, body pain, diabetes and is also used as a muscle relaxant (Jain et al., 2010; Udayan, 2004).

(c) Neem-giloe

*Neem-giloe* (*Neem-guduchi*) is *Tinospora cordifolia* plant growing on *Azadirachta indica* Guss. (*Meliaceae*) (Neem) which is also mentioned in ayurvedic literature. *Neem-giloe* has been used anti-inflammatory, immunosuppressive and hepatoprotective (Pendse, et al., 1977; Sinha et al., 2004).

Fig.3. Three different forms of *Tinospora* A. *T. cordifolia*; B. *T. sinensis*; C. *Neem-giloe*

1.6.5 Satwa

According to the ayurvedic formulary of India, ‘satwa’ is aqueous extractable solid substance collected from herbal plant (Anonymous, 2003a). In current study, we have used satwa of three *Tinospora* forms to study their hepatoprotective activity.
Table 4. Differences between *Tinospora cordifolia* and *Tinospora sinensis* (Adapted from Cooke, 1901; Narkhede et al., 2014)

<table>
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<th><em>Tinospora sinensis</em></th>
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<td>Straggling shrubs</td>
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<td>Stem</td>
<td>With lenticels</td>
<td>With lenticels</td>
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<tr>
<td>3</td>
<td>Bark</td>
<td>Green and corky</td>
<td>Dirty green, warty</td>
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<tr>
<td>4</td>
<td>T. S. of stem</td>
<td>Wheel like shape,</td>
<td>Wheel like shape,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shows white exudates</td>
<td>Shows yellow exudates</td>
</tr>
<tr>
<td>5</td>
<td>Taste</td>
<td>Bitter</td>
<td>Bitter</td>
</tr>
<tr>
<td>6</td>
<td>Leaves size</td>
<td>5.0-8.5 cm. One leaf</td>
<td>8-12 cm. More than one leaf arises from one node</td>
</tr>
<tr>
<td>7</td>
<td>Leaf proportion</td>
<td>Broad as long</td>
<td>Long as broad or broader than long</td>
</tr>
<tr>
<td>8</td>
<td>Leaves shape</td>
<td>Ovate reniform</td>
<td>Ovate of cordate</td>
</tr>
<tr>
<td>9</td>
<td>Leaf hair</td>
<td>Non hairy</td>
<td>Dense hairy</td>
</tr>
<tr>
<td>10</td>
<td>Leaf width</td>
<td>Thin papery</td>
<td>Thick leathery</td>
</tr>
<tr>
<td>11</td>
<td>Leaf colour</td>
<td>Dark green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>12</td>
<td>Leaves number</td>
<td>More (up to 10 per feet)</td>
<td>Less (Up to 4 per feet)</td>
</tr>
<tr>
<td>13</td>
<td>Petiole</td>
<td>3-4 cm long</td>
<td>8-11 cm long</td>
</tr>
<tr>
<td>14</td>
<td>Branches</td>
<td>Wiry long</td>
<td>Thickly short</td>
</tr>
<tr>
<td>15</td>
<td>Flowers</td>
<td>Greenish – yellow</td>
<td>Greenish-yellow</td>
</tr>
<tr>
<td>16</td>
<td>Flower size</td>
<td>5-8 mm across</td>
<td>5-7 mm across</td>
</tr>
<tr>
<td>17</td>
<td>Flowers male</td>
<td>Fascicled</td>
<td>Petals obovate, cuneate, rounded at the apex, not embracing the stamens</td>
</tr>
<tr>
<td>18</td>
<td>Flowers female</td>
<td>Solitary or in raceme</td>
<td>In raceme from bare branches</td>
</tr>
<tr>
<td>19</td>
<td>Drupe size</td>
<td>5-6mm across</td>
<td>0.9-1.2mm across</td>
</tr>
<tr>
<td>20</td>
<td>Drupe colour</td>
<td>Drupe orange–red when ripe</td>
<td>Drupe orange–red when ripe</td>
</tr>
<tr>
<td>21</td>
<td>Drupe shape</td>
<td>Globose</td>
<td>Ellipsoid</td>
</tr>
<tr>
<td>22</td>
<td>Flower and fruiting time</td>
<td>January – August</td>
<td>January-May</td>
</tr>
<tr>
<td>23</td>
<td>First botanically identified in India</td>
<td>1806</td>
<td>1934</td>
</tr>
<tr>
<td>24</td>
<td>Strach content</td>
<td>More</td>
<td>Comparatively poor</td>
</tr>
</tbody>
</table>
1.6.6 Nutritional Supplements

It is very important for patients with liver disease, to have balanced diet with suitable calories, carbohydrates, fats and proteins (Worman, 1999). Balanced diet with good nutritive value helps in regeneration of liver cells. Dietary supplements contain herbal products, vitamins, minerals, and any product that is not a drug (medication) (American Cancer Society, 2015). Several Asian nations use numerous food and nutrition supplements, in routine diet that possess hepatoprotective activity. Several phytochemicals present in nutritional supplements possess potential ability to prevent or reverse different kinds of liver injuries (Shukla and Kumar, 2013). Omega-3 fatty acids are also known to offer significant benefits as nutritional supplement or dietary supplement for hepatoprotection.

1.6.6.1 Polyunsaturated Fatty Acid (Omega-3 Fatty Acids)

Ancient people consumed food containing a lot more omega-3 fatty acids than we do today. Omega-3 fatty acids are characterized by double bond (C=C) between third and fourth carbon atom from methyl end of the carbon chain (Scorletti and Byrne, 2013). α-linolenic acid (ALA), found in plant oils and eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), both commonly found in marine oils, are three physiologically important omega-3 fatty acids (Rodriguez-Leyva et al., 2010). Plant oils mainly contain ALA and it is obtained from walnut, edible seeds, clary sage seed oil, algal oil, flaxseed oil, Sacha Inchi oil, Echium oil, and hemp oil (Simopoulos, 2008). EPA and DHA are chief components of fish oils, egg oil, squid oils, and krill oil (Demark-Wahnefried et al., 2001). Omega-3: Omega-6 fatty acids ratio in wild animals is 1:1 which is ideal for normal biological processes (Simopoulos, 2008).

1.7 Animal Models for Hepatotoxicity

In *in vivo* experimental models, animals are administered with repeated dose of hepatotoxin to induce liver damage. To evaluate hepatoprotective ability of drugs, various hepatotoxins (chemical agents) such as carbon tetrachloride, acetaminophen, alcohol, are used in experimental models.
1.7.1 Carbon Tetrachloride Induced Hepatotoxicity

Formerly, carbon tetrachloride (CCl₄) was used in fire-extinguishers, as a grain fumigant and also as a refrigerant (El-Sayed et al., 2015). Carbon tetrachloride (CCl₄) is one of the commonly used model drug (hepatotoxin) to induce hepatotoxicity in experimental models to study acute and chronic liver failures (Singh et al., 2012; Olatosin et al., 2014). In endoplasmic reticulum and mitochondria, cytochrome P-450 metabolizes CCl₄ and forms a reactive oxidative free radical called CCl₃O which induces lipid peroxidation (Weber et al., 2003). Within 3 hours of single dose of CCl₄ administration, poison reaches its maximum concentration, and within 24 hours centrilobular necrosis and fatty changes are observed in experimental rats (Singh et al., 2012). Different methods and concentrations of doses used in experimental models are: repetitive dose of 0.1 to 3ml CCl₄/kg bw, I.P. for 7 days; 1 ml CCl₄/kg bw, I.P in liquid paraffin (30% v/v); 1ml CCl₄/kg bw, I.P. single dose to induce acute hepatotoxicity (Adewale et al., 2014; Sarkar et al., 2014).

1.7.2 Acetaminophen Induced Hepatotoxicity

Acetaminophen (international name used in USA and Japan) and Paracetamol (international name used in Europe) are two official names of the same chemical compound derived from its chemical name: N-acetil-para-aminophenol (Benista and Nowak, 2014). Acetaminophen is over-the-counter analgesic and anti-pyretic medicine. Acetaminophen is a dynamic metabolite of phenacetin used to get relief from fever, migraine, muscle hurts, joint inflammation, spinal pain, toothache and frosty (Vidhya Malar and Bai, 2012). Overdose of acetaminophen leads to ‘Acetaminophen hepatotoxicity,’ causes liver injury and is one of the most common causes of poisoning all over world (Vidhya Malar and Bai, 2012). Therapeutic dose of acetaminophen is safe but, its overdose can cause hepatotoxicity and acute liver failure (Michaut et al., 2014). Higher dose of acetaminophen causes hepatotoxicity in human and animal models (Jaeschke et al., 2014). Events of acetaminophen hepatotoxicity lead to liver cirrhosis, hepatitis etc. (Fontana, 2008).

It is primarily metabolized by sulfation and glucuronidation to unreactive metabolites, and then activated by cytochrome P450 system. Bioactivation of acetaminophen produces a toxic electrophile, N-acetyl p- benzoquinone imine
NAPQI. NAPQI binds covalently to tissue macromolecules and also induces lipid oxidation. Apart from oxidizing lipids, NAPQI also oxidizes sulphhydryl groups in protein thiols. NAPQI is also known to alter the homeostasis of calcium (Lin et al., 1997). Different reactive metabolites are produced during acetaminophen metabolism, which covalently modify proteins (Bernareggi, 1998), impose oxidative stress (Ritter and Giganti, 1998) and results in mitochondrial injury (Mingatto et al., 2000). Several studies in animals and human have demonstrated that paracetamol overdose causes liver damage primarily due to enhanced production and/or decreased glutathione conjugation of NAPQI that eventually results in increased covalent binding of NAPQI to cell proteins (Leung et al., 2012; Sharoud, 2015). Several experimental studies reveal that daily treatment of acetaminophen to Wistar albino rats [at a dose of 600mg/kg for 14 days (Ita et al., 2009) or a single dose of 500 mg/kg to 3 gm/kg (Murugesh et al., 2005; Juraj et al., 2004) or a single dose of 2g/kg (Meganathan et al., 2011; Prabu et al., 2011) or a dose of 400 mg/kg for seven days (Kanchan and Sadiq, 2011)] leads to liver injury and consequent increase in the levels of liver marker enzymes.

1.7.3 Alcohol Induced Hepatotoxicity

Liver is among the organs most susceptible to the toxic effects of ethanol. Alcoholic liver disease (ALD) is considered a major health and economic problem worldwide (Bruha et al., 2012; Cui et al., 2013). Alcohols are hydroxy derivatives of aliphatic hydrocarbons and commonly consumed alcohol is ethyl alcohol or ethanol (Tripathi, 2013). Alcohol is a psychoactive and addictive substance which is quickly absorbed by the body and detoxified by liver (Hadzic et al., 2013). Alcohol (Ethanol) is one of the most important and commonly used hepatotoxic agents in the experimental study of liver related disorders. The alcohol over dose leads to liver damages (Arulkumaran et al., 2009) caused by complex mechanisms involving metabolites of ethanol with ability to form protein adducts with several proteins of hepatocytes (Zimmerman, 1999). Further, it has direct cytotoxicity which leads to increase in reduced form of nicotinamide adenine dinucleotide (NADH) causing fat accumulation (Zimmerman, 1999). Many pathways are reported to be involved in ALD, including oxidative stress and mitochondrial damage (Stewart et al., 2001; Wu and Cederbaum, 2003; Gramenzi et al., 2006). Ethanol is metabolized in the body by enzyme catalyzed oxidative processes into acetaldehyde. The acetaldehyde is further
oxidized to acetate which is then converted to carbon dioxide via the citric acid cycle (Samundeeswari et al., 2013). Alcohol metabolites also cause induction of free radicals leading to peroxidation and inflammatory response (Jarvelainen, 2000). Various different concentrations of alcohol at different doses have been reported to be hepatotoxic in animal studies. Alcohol (15% to 40%) at a dose of 2ml-25 ml/kg bw or 1.5gm-24gm/kg bw (Mahendran and Devi, 2001; Ghosh et al., 2007; Arulkumaran et al., 2009; Patel et al., 2010; Nigam and Paarakh, 2011; Radhika et al., 2011; Singh and Gupta, 2011; Vetriselvan et al., 2011; Sudhir et al., 2012) is reported to induce hepatic damage in Wistar rats.

In recent years, herbal medicine and nutritional supplementation have been used as alternative medicine to treat liver disorder (Lee et al., 2007; Abo El-Magd et al., 2015). *Tinospora* is known to be used in many ailments in alternative medicine. Omega 3 fatty acids are also known to have several health benefits (Wu et al., 2012; Li et al., 2014). In current study, protective, corrective and prophylactic effects of Guduchi satwa and omega-3 fatty acids against acetaminophen and alcohol induced hepatotoxicity were analyzed.

1.8 Genesis of Thesis

Herbal medicines and nutrition are known to play an important role in management of various health ailments. Acetaminophen and alcohol induced hepatotoxicity may be modulated through interventions with herbal and nutritional supplements. The efficacy of the interventions can be enhanced by their simultaneous delivery during progression of hepatotoxicity and may serve as curative as well as preventive therapies against liver toxicity.

In this study three different forms of *Tinospora* i.e. *T. cordifolia, T. sinensis* and *Neem-giloe* were analyzed for the hepatoprotective efficacy of their satwa. *T. cordifolia* is easily available in the fields and hence it is used frequently. But it is observed that the description of Guduchi in Ayurvedic literature matches with *T. sinensis*. Hence, to ascertain the most potent variant of Guduchi, hepatoprotective activity of all three satwa (from *T. cordifolia, T. sinensis* and *Neem-giloe*) was assessed against acetaminophen and alcohol induced hepatotoxicity in rats.
In this study, flax oil and fish oil were used as nutritional supplements (rich source of omega-3 fatty acids) to assess their hepatoprotective activity against acetaminophen and alcohol induced liver toxicity. The protective and corrective effects of nutritional and herbal interventions against acetaminophen and alcohol induced hepatotoxicity in rats were also studied.

Hypothesis

Pathophysiology of acetaminophen and alcohol induced hepatotoxicity may be modulated through interventions with herbal and nutritional supplements. The efficacy of the interventions can be enhanced by their simultaneous delivery during progression of hepatotoxicity and may have corrective/preventive roles in liver toxicity.