Introduction
1. INTRODUCTION

The liver plays a vital role in bodily disposition of drugs. By virtue of its strategic location as the delta of portal blood stream, it is massively exposed to drugs and other foreign compounds, absorbed through the intestine. Hepatic uptake, biotransformation, intracellular transport and excretion in bile are important processes that clears the drug in the body, terminate their pharmacological action and prevent their toxic accumulation (McLean and Morgan, 1991). However, drugs induced hepatotoxicity results in deviation from normal structure and function of liver and it may lead to necrosis, cirrhosis, carcinoma or fatty infiltration, accompanied by biochemical and physiological changes in this organ. Severe liver injury is reported to emanate from administration of therapeutic doses of acetaminophen, antiepileptic drugs, cinchophane, halothane, contraceptive steroids and antitubercular drugs (Mahendale, 1985; Zimmerman and Maddrey, 1993; Chitturi and Farrell, 2000). Hepatic necrosis and other hepatocellular damage can be produced by the direct action of the drug itself or through their metabolites. Activation of toxic substances in the liver is an established phenomenon that has been identified as the cause for several classes of hepatotoxicity. Drugs induced hepatotoxicity is a major concern because of the risks involved and the purpose of use of potentially hepatotoxic drugs in patients (Mahendale, 1985).

In the chemotherapy of tuberculosis, Isoniazid and Rifampicin have been termed as “First Line” drugs and have found worldwide application.
Presently, Pyrazinamide is also administered with these drugs in the short-term chemotherapy of tuberculosis (Petri, 2001). In view of their high efficacy and wide spread application, the above antitubercular drugs are employed in the chemotherapy of tuberculosis. However, since hepatotoxicity on continued administration of antitubercular drugs is now being increasingly realized in clinical practice, protection against these drugs induced hepatotoxicity is warranted. Hepatic necrosis, hepatitis, jaundice and hepatic steatosis are different facets of antitubercular drugs induced hepatotoxicity and a hepatoprotective agent that could prevent these pathological manifestations without themselves producing any undesirable effects is needed for its use in clinical practice. Hence, search for identification of new principles extracted from plants that could effectively prevent the hepatotoxicity induced by these agents is presently underway.

In this study, the plant principles extracted from the leaf of the herbal medicinal plant, Cassia fistula Linn. has been tested for their efficacy against antitubercular drugs induced hepatotoxicity. Silymarin, a standard hepatoprotective agent, employed in the treatment of alcoholic liver disease is also tested for its efficacy on these grounds.

1.1 Therapeutic implication of antitubercular drugs

Prevention of tuberculosis is a major strategy in disease control in U.S. and in other countries. The WHO has cautioned about the fast increasing number of tuberculosis patients worldwide (McCray et al., 1997; Zuber et al., 1997). It is now estimated that more than 4 million persons worldwide are
suffering from active forms of tuberculosis. Further, increasing number of HIV positive cases and their co-infection with tuberculosis has necessitated the increased utility of antitubercular drugs in the chemotherapy of tuberculosis (Whalen et al., 1997; Halsey et al., 1998). Ironically, even 40 years of its introduction, isoniazid preventive therapy for tuberculosis infection is still a subject of debate mainly because of the concern about the hepatotoxicity associated with isoniazid and other antitubercular drugs (Colice, 1990; Jordan et al., 1991; Isrel et al., 1992; Sarasin et al., 1995). A base line survey of data conducted for the past 20 years shows the evidence of antitubercular drugs induced hepatotoxicity in 5 to 20 cases per 1000 persons (Garibaldi et al., 1972; Nolan et al., 1999).

1.2 Isoniazid

Isonicotinic acid hydrazine (Isoniazid, INH) is still considered to be the drug of “First Choice” for the chemotherapy of tuberculosis.

1.2.1 History

Evans (1968) reported that 1-isonicotinyl hydrazine was first synthesized by Meyer and Mally in 1912, but its chemotherapeutic value was not discovered until 40 years later. INH is also said to be discovered independently and simultaneously at three separate centers. It is said that Lotf and co-workers at Squibb laboratories (Bernstein et al., 1952) and Fox at Hoffman-La Roche (Fox, 1952) discovered the drug by different pathways. Domagk, in a communication at the Congress of an Internal Medicine in Wiesbaden, Germany in April 1952, claimed that isonicotinic acid hydrazid
was synthesized by Offe and tested by Domagk as early as 1950s in Farbenfabriken, Bayer in Germany. The interesting history of these chemical studies has been reviewed by Fox (1952). However, discovery of INH is said to be somewhat fortuitous. It was identified that thiosemicarbazones inhibit *Mycobacterium tuberculosis* and hence, the thiosemicarbazones of isonicotinylaldehyde was synthesized and studied. The starting material for the synthesis was the methyl esters of isonicotinic acid and the first intermediate was isonicotinyl hydrazine (Mandell and Sande, 1985).

### 1.2.2 Chemistry

INH is the hydrazide of isonicotinic acid. It is a simple molecule (M.W. 137), freely soluble in water and its structural formula is as follows:

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\text{CONH NH}_2
\]

Isoniazid

### 1.2.3 Antibacterial activity

Grunberg and Schnitzer (1952) reported that INH has a bacteriostatic action against *Mycobacterium tuberculosis*. The reports of antituberculous activities of INH in mice (Grunberg and Schitzer, 1952), guinea pigs and rabbits (Steenkem and Wolinsky, 1952) has thrown light on its efficacy against tuberculosis. The first report of its effectiveness in human patients
(Robitzek and Selikoff, 1952) showed that clinically also it is more powerful against both pulmonary and non-pulmonary tuberculosis. From these reports, the efficacy of treatment of INH has been increasingly realized. INH is bacteriocidal for rapidly dividing microorganisms and is bacteriostatic to resistant bacilli. It inhibits the biosynthesis of mycolic acid, which is an important constituent of bacterial cell wall. The \( \text{inhA} \) gene is said to be the primary target of this drug (Vilcheze et al., 2000).

1.2.4 Absorption, distribution and biotransformation

INH is rapidly absorbed when administered orally and it attains peak plasma concentration of 3 to 5 \( \mu \text{g/ml} \) within 1 to 2h after oral ingestion in human. Aluminium containing antacids retard its rate of absorption. INH diffuses rapidly to all body fluids and is detectable in significant quantities in pleural ascitic fluids and in CSF. 75 to 95\% of dose of INH is said to be excreted in urine within 24h as its metabolites. During metabolism, INH is said to be acetylated (by the enzyme n-acetyl transferase) to acetylisoniazid, which on further enzymic hydrolysis forms isonicotinic acid (a non-toxic metabolite of INH) leading to its excretion in the urine (Nelson et al., 1976a,b; Timbrell et al., 1977; Whitehouse et al., 1983; Petri, 2001). On the other hand, acetylisoniazid was also shown to be further biotransformed by cytochrome P450 enzymes in the liver to release highly reactive hepatotoxic metabolites, acetylhydrazine (Whitehouse et al., 1983) and hydrazine (Sarich et al., 1995).
1.2.5 Acetylation phenotype and INH toxicity

Human and animal population show genetic heterogeneity with regard to the rate of acetylation of INH. Three acetylator phenotypes namely, slow, rapid and intermediate acetylators seem to exist in human and this status is said to be genetically determined (Evans et al., 1960; Evans, 1968). The rate of acetylation significantly alters the concentration of INH and its metabolites that are achieved in plasma and their half-life in circulation. Fast acetylators of INH are found in Japanese population and slow acetylator phenotype among Scandinavians, Jews and North and South African Caucasians (Petri, 2001). It is said that the slow acetylators are prone for INH induced hepatotoxicity than the rapid acetylators (Mitchell et al., 1976). However, studies have shown that acetylator phenotype is not determinable for INH induced hepatotoxicity in human (Gurumurthy et al., 1984; Singh et al., 1995).

1.2.6 Therapeutic use

INH is commonly administered at a daily dose of 5 mg/kg with the maximum of 300 mg as oral and intramuscular dose. Children should receive 10 to 20 mg/kg per day (300mg maximum). Pyridoxine (15 to 50 mg/day) should be administered with INH to minimize peripheral neuritis in malnourished pediatrics, HIV infected individuals, diabetics, alcoholics and uremics (Snider, 1980).
1.2.7 Untoward effects

The incidence of adverse reactions to INH was estimated to be 5.4%. The prominent are the rashes (2%), fever (1.2%), jaundice (0.6%) and peripheral neuritis (0.2%). Pyridoxine is advocated concomitantly with INH to prevent peripheral neuritis, which is a common untoward effect in patients receiving 5 mg of this drug daily. Attempted suicide with INH results in nausea, vomiting, dizziness, slurred speech, visual hallucinations, followed by coma, seizures, metabolic acidosis and hyperglycemia (Petri, 2001). The most prominent adverse reaction to INH is the development of hepatotoxicity manifested as hepatic necrosis or steatosis or a combination of both in human and experimental animals (Mitchell et al., 1976; Timbrell, 1979; Whitehouse et al., 1983; Karthikeyan and Krishnamoorthy, 1991; Sarich et al., 1995).

1.3 Rifampicin

1.3.1 History

Rifampicin (RIF) was developed by the research laboratory of Lepetit S.P.A. in Milan, Italy in 1963 (Furesz and Timber, 1963). Its efficacy against Tubercle bacilli was tested in France (Canetti et al., 1968), U.S (Hobby and Lenert, 1968), Europe and South America (Baronti and Lukinovich, 1968; Gyseien et al., 1968). The Chemical Industry of Basal (CIBA) made available RIF as RAMICITENE® for controlled trial for patients in U.S Public Health Service, Tuberculosis Therapy Trials. This initial experience suggested RIF as a broad spectrum antibiotic with far reaching impact for the treatment of tuberculosis in U.S (Newman et al., 1971).
1.3.2 Chemistry

The Rifamycins (Rifampin, Rifamycin, Rifampicin) are group of structurally similar, complex macrolytic antibiotics produced by *Streptomyces mediterranei*. RIF is a semi synthetic derivative of one of these Rifamycin B. RIF is a zwitter ion having M.W. of 823. It is soluble in organic solvents and water at acidic pH. It has the following structure:

![Rifampicin structure](image)

1.3.3 Antibacterial activity

RIF inhibits the growth of most gram positive as well as many gram negative bacteria. It inhibits the growth of *Mycobacterium tuberculosis* at a concentration of 0.005 to 0.2 µg/ml in culture studies. RIF inhibits DNA dependent RNA polymerase, leading to the suppression of initiation of chain formation in RNA synthesis. RIF is bactericidal for both intracellular and extracellular micro organisms (Wehrli, 1977).
1.3.4 Absorption, distribution and biotransformation

Oral administration of RIF produce a peak plasma concentration 2h after ingestion of 600 mg, and about 75 to 80 percent of RIF in circulation is bound to plasma proteins (Mandell and Sande, 1985). After absorption from gastro intestinal tract, RIF is rapidly eliminated into the bile, which is increased in the presence of hepatic dysfunction and it is decreased in patients receiving concomitantly INH, who are slow acetylators of this drug. The half life of RIF is progressively shortened by about 40% during the first 14 days of treatment due to induction of microsomal enzymes, with acceleration of deacetylation of the drug (Petri, 2001).

RIF is distributed throughout the body and is present in effective concentrations in many organs and body fluids including CSF. This is perhaps accompanied by the fact that the drug may impart an orange red colour to the urine, feces, saliva, sputum, tears and sweat (Furesz, 1970; Farr, 2000).

1.3.5 Therapeutic use

RIF is available alone and as fixed dose combination with INH. The dose of RIF recommended for treatment in tuberculosis in adults is 600 mg, given once a day. Children should receive 10 mg/kg. RIF, like INH should never be used alone because it rapidly develops resistance to *Mycobacterium tuberculosis* (Petri, 2001).
1.3.6 Untoward effects

The occurrence of side effects of RIF is dependant on combination in which it is given, especially in the combination of RIF and INH. When given in usual doses, less than 4% of patients with tuberculosis develop significant adverse reactions characterized by rash (0.8%), fever (0.5%), nausea and vomiting (1.5%) (Grosset and Leventis, 1983), gastro-intestinal disturbances such as epi-gastric distress, nausea and vomiting, abdominal cramps and nervous system related symptoms such as fatigueness, drowsiness, headache, dizziness, ataxia, confusion, muscular weakness and hypersensitivity reactions such as pruritus, skin eruptions, eosinophilia and roughness of mouth and tongue. Since, the potential teratogenicity of RIF is unknown, and the drug is known to cross placenta, it is best to avoid the use of this drug during pregnancy (Petri, 2001).

1.4 Pyrazinamide

1.4.1 Chemistry

Pyrazinamide (PYR) is a synthetic pyrazine analog of nicotinamide. It is a small molecule having a M.W. of 123.1 and its structure is as follows:

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Pyrazinamide
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\text{Pyrazinamide}
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1.4.2 Antibacterial activity

PYR inhibits the growth of bacteria at acidic pH. Since, *Mycobacterium tuberculosis* resides in phagosomes within the macrophages, it readily kills the growth of this bacteria (Jacobs, 2000). The target of PYR appears to be the mycobacterial fatty acid synthase I gene, which is essential for the synthesis of mycolic acid, a component of the bacterial cell wall (Zimhony *et al.*, 2000). Resistance to bacteria develops when PYR is used alone.

1.4.3 Absorption, distribution and biotransformation

PYR is well absorbed from gastro-intestinal tract and is widely distributed throughout the body. The plasma peak concentration is achieved in 2h and its half-life is 9 to 10h. PYR is distributed widely, including the CNS after oral administration and it is excreted in the urine by renal glomerular function. PYR is hydrolysed to pyrazinoic acid and is subsequently hydroxylated to 5-hydroxy pyrazinoic acid, which is the major excretory product (Petri, 2001).

1.4.4 Therapeutic use

PYR is an important component of short-term (6 months) multi drug chemotherapy of tuberculosis (British Thoracic Association, 1983; Bass *et al.*, 1994). The daily dose recommended for human is 75 to 300 mg orally and the maximum quantity that can be administered is 2 gm per day regardless of weight. Children should also receive the same dose (Petri, 2001).
1.4.5 Untoward effects

Injury to the liver is the most serious side effect of PYR. The signs and symptoms of hepatic disease appear in about 15 percent of patients on oral administration of 40 to 50 mg of PYR and 2 to 3 percent death may occur due to necrosis in rare incidences (McDermott et al., 1954). The other untoward effects observed with PYR are arthralgias, anorexia, nausea and vomiting, dysuria and fever (Petri, 2001).

1.5 Hepatotoxicity of antitubercular drugs treatment in human

Since its introduction in 1952, INH has been widely used in the treatment of tuberculosis, either alone or in combination with one or more antitubercular drugs such as RIF, PYR, streptomycin, amino salicylic acid and ethambutol. Though an efficacious agent against tuberculosis, administration of INH along with other antitubercular drugs has been shown to induce jaundice, resulting in fulminant, submassive necrotic hepatitis, hepatic coma and death in various clinical studies conducted between 1952 to 1960 (Randolph and Jones, 1953; Gellis and Murphy, 1955; Paine, 1958; Merritt et al., 1959; Cohen et al., 1961). Scharer and Smith (1969) reported onset of asymptomatic abnormalities, signaled by elevation of transaminases in the first month of INH chemotherapy in about 10% of recipients of antitubercular drugs. Maddrey and Boitnott (1973) observed the development of INH induced hepatitis in human. The frequency of INH induced hepatotoxicity is highly variable in human, ranging from 8.7 to 23% (Bailey et al., 1974;
Mitchell et al., 1976) and it is said to be manifested as mild or transient elevation in the activities of transaminases, ALP and Bilirubin in serum, which sets in between 2 to 6 months after initiation of INH therapy.

Hypersensitivity reactions and prominent liver cell necrosis, accompanied by hepatitis were also reported in various clinical studies (Lees et al., 1971; Scheuer et al., 1974; Black et al., 1975). The incidence of clinical hepatitis in patients receiving INH and RIF was reported in 5 to 8% of the recipients and 1% in persons treated with INH alone (Lees et al., 1971). Development of high incidence of jaundice (Lal et al., 1972) and fulminant hepatitis in adults (Pessayre et al., 1977) and in children (Ramachandran, 1980) has been reported in persons receiving chemotherapeutic doses of INH and RIF. Biochemical evaluations showed elevated levels of transaminases and alkaline phosphatase in children, indicating the development of hepatotoxicity (Ramachandran, 1980; Linna and Uhari, 1980; Thulasimani, 1982; Rugmini and Mehta, 1984) on administration of INH and RIF.

PYR is implicated commonly in short-term chemotherapy of tuberculosis and it is administered along with INH and RIF. This combined antitubercular therapy has been reported to induce symptoms of hepatotoxicity in 15% of patients of which nearly 2 to 3% develop jaundice (Zierski and Bek, 1980; Pilheu et al., 1981; Dutt and Stead, 1982). The reports clearly demonstrate the hepatotoxic potential of the above “first line” antitubercular drugs, administered either alone or in combination with each other in human.
1.6 Antitubercular drugs induced hepatotoxicity in animal models

Administration of INH and or its metabolites has been reported to cause hepatotoxicity in dogs (Rubin and Burke, 1953), rabbits (Yard and McKennis, 1955; McKennis et al., 1955), guinea pigs (Raisfeld, 1975; Heisey et al., 1980) and rats (Snodgrass et al., 1974; Mitchell and Jollows, 1975; Mitchell et al., 1976; Timbrell et al., 1977). Phenobarbital pretreatment was demonstrated to induce hepatocellular necrosis and fatty liver on administration of INH, acetylhydrazine and acetylisoniazid (Mitchell et al., 1976; Wright and Timbrell, 1978; Timbrell et al., 1980; Whitehouse et al., 1983; Krishnamoorthy and Karthikeyan, 1991). Fatty infiltration of the liver on INH administration was reported in dogs (Rubin et al., 1952) and rabbits (McKennis et al., 1955; Whitehouse et al., 1983; Karthikeyan and Krishnamoorthy, 1991; Sarich et al., 1995).

Hepatotoxicity, manifested as hepatic cellular necrosis was shown in young rats treated with INH and RIF (Sodhi et al., 1997). Combined administration of these antitubercular drugs increased the levels of LPO and decreased the activities of CAT and SOD in the liver tissue, indicating hepatocellular damage in rats (Prabakan et al., 2000). Increase in the activity of transaminases, bilirubin, ALP and total protein, accompanied by histopathological changes such as hepatocellular degeneration, necrosis and fibrosis in the liver tissue was reported in rats treated with INH, RIF and PYR (Kale et al., 2003). Increase in the levels of CHO, TG, FFA, TL and LPO in the liver tissue and elevated levels of transaminases, ALP and bilirubin in serum with histopathological changes such as dilated sinusoids, portal
inflammation and microvesicular fatty changes were shown in rats treated with INH+RIF+PYR (Pari and Ashokkumar, 2002). Alteration in cytochrome P450 enzymes and accumulation of calcium in the liver associated with increase in LPO and decrease in the activities of CAT and GPx in the liver tissue were reported in rats treated with the above antitubercular drugs (Tasduq et al., 2005). The above findings clearly demonstrates the hepatotoxic potential of INH, administered either alone or in combination with other antitubercular drugs in experimental animals.

1.7 Steatosis (or) Fatty infiltration of the liver

Abnormal accumulation of fat in the parenchymal cells of liver tissue is a common response of the liver to injury and this pathological condition is often referred as “steatosis” or “fatty infiltration” or “fatty metamorphosis”. During steatosis, fat droplets accumulate in the cytoplasm of the hepatic parenchymal cells. Macroscopically, the liver is grossly enlarged, smooth and pale during steatosis (Lombardi, 1965). Fatty liver occurs due to an imbalance between the rate of synthesis and the rate of utilization of hepatic triglycerides in the liver and this effect could be brought about by the administration of certain drugs and chemicals such as alcohols, tetracyclins, corticosteroids, CCl₄ etc. (Dianzani, 1979). Non-alcoholic steatohepatitis (NASH) is an important pathological condition because it may progress further, leading to cirrhosis or fibrosis of the liver with fatal outcome. The histological features of NASH include, macrovesicular steatosis, nuclear glycogenation, lobular and portal inflammation, which resembles those associated with alcohol induced liver injury (Malaguarnera et al., 2005).
1.8 Antitubercular drugs induced hepatic steatosis

INH induced fatty infiltration of the liver was first reported in dogs (Rubin et al., 1952). In human, fatty infiltration of kidney, liver and heart was reported in two cases of fatal poisoning induced by high dose of INH (Camba, 1953). McKennis et al. (1955) showed that administration of an acute dose of INH (100 mg/kg, i.p) could induce fatty infiltration in liver of rabbits. Karthikeyan (2002) reported that an acute dose of INH (150 mg/kg), could precipitate fatty infiltration of the liver, associated with an increase in the levels of various lipid parameters (TL, TG, CHO, FFA and PL) in plasma of rabbits. Hypertriglyceridemia, indicative of hepatic steatosis was also reported in rabbits administered an acute dose of INH (Sarich et al., 1995, 1996). Whitehouse et al. (1983) demonstrated the induction of hypertriglyceridemia and steatosis in the liver of rabbits administered with a sub-acute dose of INH (50 mg/kg for 11 days) and its prevention by simultaneous treatment of Vit. B6, and this effect was confirmed by later workers (Karthikeyan and Krishnamoorthy, 1991). Pretreatment of rabbits with phenobarbitone was shown to precipitate steatosis in the liver tissue of rabbits treated with INH (Krishnamoorthy and Karthikeyan, 1991). Pari and Ashokkumar (2002) reported an increase in the levels of CHO, TG, FFA and TL in the liver tissue of rats treated with INH, RIF and PYR.

1.9 Herbal remedies as an alternative for drugs induced hepatotoxicity

Traditionally, herbal medicines have been used worldwide as an alternative in health care systems and this practice is considered in general, as
non-toxic and even harmless because of their natural origin (WHO, 1995; Sheldon et al., 1997; Deng et al., 1997). Ayurvedha and Siddha medicinal practices have been in existence for over 5000 years and these alternative medicinal practices have been shown to cure various ailments including liver disorders and jaundice (Chopra et al., 1992). Medicinal plants are still considered as the source of untrapped reservoir of drugs, and the structural diversity of the component molecules make them valuable sources of novel component against various drugs and chemicals induced hepatotoxicity (Farnsworth, 1989). Recently, renewed interest has been focused on screening thousands of medicinal plants, mainly for the purpose of discovery of novel compounds of pharmaceutical value with hepatoprotective and antioxidant properties. With this objective in mind, several plants used in traditional system of medicine have been screened for their efficacy against drugs and chemicals induced hepatotoxicity in various experimental animals. The hepatoprotective, antioxidant and the free radical quenching properties of various medicinal plants such as Solanum nigrum, Cichorium intybus (Sultana et al., 1995), Picrorhiza kuroa, Curcuma longa, Camellia sinensis and Silybum marianum (Luper, 1998) have already been investigated against various drugs and chemicals induced hepatotoxicity.

Since liver toxicity has been reported in about 10 to 20 percent of recipients of INH in human (Mitchell et al., 1976; Nolan et al., 1999) as well as in experimental animals as an adverse reaction observed during administration of antitubercular drugs, attempts have been made by few
workers to test the efficacy of herbal remedies as an alternative for hepatoprotection against these drugs. The review of literature on these grounds is presented here under.

1.10 **Antitubercular drugs induced hepatotoxicity and its prevention by herbal medicinal preparations in experimental animals**

The concept of finding a herbal remedy against antitubercular drugs induced hepatotoxicity is fairly recent and only limited literature is available on these grounds. INH alone induced hepatotoxicity as indicated by an increase in LPO and decrease in glycogen in the liver of rats was shown to be prevented by simultaneous administration of HD-03, a poly herbal formulation containing *Solanum nigrum, Cichorium intybus, Picrorhiza kuroa, Tephrosia purpurea* and *Andrographis paniculata* (Mitra *et al*., 1998). Administration of Liv. 100, a poly herbal medicinal formulation, was shown to protect the liver against antitubercular drugs induced hepatotoxicity in rats (Saraswathy *et al*., 1998). Increase in LPO and decrease in the activities of SOD and CAT in the liver tissue of rats induced by administration of INH and RIF was shown to be completely prevented by administration of ethanolic leaf extract of *Hemidesmus indicus* and this effect was attributed to the free radical quenching properties of coumarino lignoids namely, hemidesmin – 1 and hemidesmin – 2, present in the extract (Prabakan *et al*., 2000). Antitubercular drugs induced hepatotoxicity, indicated by an increase in transaminases, ALP and bilirubin in serum and increase in lipid parameters (CHO, TG, FFA and TL) and LPO in the liver tissue of rats was shown to be
prevented by simultaneous administration of ethanolic leaf extract of *Moringa oleifera* (Pari and Ashokkumar, 2002). The mono methyl fumarate, isolated from *Fumaria indica*, was shown to protect the liver against RIF induced hepatotoxicity in rats (Rao and Mishra, 1998). The aqueous extract of *Azadirachta indica*, was reported to prevent antitubercular drugs induced hepatotoxicity in rats (Kale et al., 2003).

Though, the above studies demonstrate noticeable hepatoprotective and antioxidant properties against antitubercular drugs induced hepatotoxicity, their efficacy and relative safety over prolonged administration in combination with the antitubercular drugs remain unknown. It is noteworthy to specify that the efficacy of these medicinal plants against INH and other antitubercular drugs induced hepatic steatosis have not been demonstrated. Hence, in this study, a novel attempt has been made to evaluate the hepatoprotective, antioxidant and antilipidemic properties of ethanolic leaf extract of *Cassia fistula* Linn. as well as Silymarin against INH and other antitubercular drugs induced hepatotoxicity using rats as animal models. The literature review presented here under describes the essential features of herbal medicinal plant *Cassia fistula* Linn. and the hepatoprotective principle Silymarin and the rationale for their selection in this study.

1.11 *Cassia fistula* Linn.

1.11.1 Description of the plant

*Cassia fistula* Linn. is a medium sized tree, widely cultivated throughout India as an ornamental and deciduous plant (Chatterjee and
Pakrashi, 1992). It is also cultivated in West Indies, Sri Lanka, China, Egypt and in some European countries. It is a fast growing tree, which reaches 30 to 40 feet in height and width. Its well spread branches are clothed with pinnately compound leaves. It bears yellow blossoms, which are pendent and racemous and are found as thick clusters, slightly drooping from the branches during summer seasons. The trunk is straight and the bark is smooth, pale gray in young plants, which becomes rough and dark brown when matured. Pods are cylindrical, pendulous, 25 to 30 cm long and 1.5 to 3 cm in diameter. The seeds are numerous, small, flat, smooth and yellowish gray in colour. The seeds in ripe pods are surrounded by black pulp. The photograph of this plant with leaves, flowers and pods is presented in Plate – 1.

1.11.2 Botanical nomenclature

The botanical nomenclature of this plant is as follows:

Kingdom : Plantae
Sub-Kingdom : Tracheobionta
Super-Division : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Sub-Class : Rosidae
Family : Caesalpinaceae
Genus : Cassia
Species : fistula
PLATE - 1

CASSIA FISTULA LINN.
1.11.3 Vernacular names of *Cassia fistula* Linn.

In India, this plant is called by different names in different local vernacular languages. *Assamese* – Sonar; *Bengali* – Sonali; *Hindi* – Amaltas, Bandarlauri; *Malayalam* – Konna; *Sanskrit* – Aragvadha; *Tamil* – Konrai, Konnai, Sarakonrai, Appai; *Urdu* – Amaltas; *English* – Cassia, Golden shower, Indian laburnum, Pudding pipe.

In different geographical locations of the world, this plant is identified by various names. *Myanmar* – Gnookye; *Sri Lanka* – Kavani; *Nepal* – Bandarlata; *Persian* – Khiyarchanbar; *China* – A Po Le, Hoa Ts’in; *France* – Casse officinale; Cane fice; *Portuguese* – Canna fistula; *Italy* – Cassia (Chopra et al., 1956).

1.11.4 Description of the leaves

The leaves are 23 to 40 cm long, main rachis, pubescent, stipules minute, linear oblong, obtuse pubescent. Leaflets 4 to 8 pairs, ovate or ovate oblong, acute, 5 to 12.5 by 3.8 to 9.5 cm, bright green and glabrous above, paler and silvery pubescent beneath when young. The midrib densely pubescent on the underside, base cuneate, main nerves numerous and conspicuous beneath. Petioles 6 to 10 mm, long pubescent or glabrous (Chopra et al., 1992).
1.11.5 Medicinal properties of *Cassia fistula* Linn.

This plant has been used in the treatment of various ailments dating back to Susrutha Samitha and Charaka Samitha. The traditional use of this plant in Ayurvedic medicine is inscribed by the following Sanskrit version:

अरघवधोति मुघर: शीत: शूलापहारकः ||
ज्वरकण्ठुकुष्ठेनविष्टम्भनाशनः ||

(राजनिघण्डु)

aragvadjoti madura: sita:sulapaharaka:.
jvara kandu kushtha mehaka phavistambhanasana:..

(rajanighantu)

which reads *Aragvadha* is madhura (sweet) sheeta (sheetaveerya – against cold), anticolic, antipyretic, beneficial in pruritus and other skin diseases, polyuria, deranged kapha (cold) and flatulence.

In Siddha medicine, the traditional use of this medicinal plant has been inscribed in the following lyrics.

cadanta

मालेन्यमनिः भीतसेवम पुष्पकयुक्तिः विनिशंकात
मालेन्यमनिः संहारकतःमुख्तिः - कराचन्तु

cadanta

कुस्तिकानीतिसमन्तो मालिकामिना हिंगाः

cadanta
Meaning of the poem in Tamil language is:

The translation of the above in English is:

The leaves of Kondrai (Cassia fistula) acts as fast as the weapon (Sudarsana Chakra) of Lord Krishna (i.e., Panduranga) to bring down the sexually transmitted diseases under control (of our hands). Moreover, you should know that it (Cassia fistula) does many help when being identified and used as medicine, for the betterment of human beings in this world.

1.11.6 Application of Cassia fistula Linn. in various disease condition

The seeds are useful in skin disorders and sore throat and it is also used for the treatment of oral sores (Bodding, 1983). In Ayurvedic medicine, this plant has been used for the treatment of hematemesis, pruritus, leucoderma and diabetes. Ethanolic extract of the pods and stem bark are said to exhibit hypoglycemic, antiviral and anticancer properties. This plant is also reported to be used in the treatment of cancer, epilepsy, convulsions, delirium fibris, pimples, burns, syphilis and dysuria. The leaves are said to be useful in ringworm infection and flowers are reported to be effective in fungal infection.
(Chopra et al., 1956). In Sri Lanka, this plant is said to be used in the treatment of skeletal fractures (Ekanayake, 1980).

1.11.7 Hepatoprotective properties of the leaf extract of *Cassia fistula* Linn.

Bhakta et al. (1999) learned that the urban people of North Eastern regions of India use the pods and the leaves of the *Cassia fistula* Linn. as antiallergic and hepatoprotective agent. This claim of traditional use of this plant as hepatoprotective agents led these investigators to evaluate the hepatoprotective effect of the leaf extract of this plant against CCl₄ and paracetamol induced hepatotoxicity. Administration of 400 mg/kg of n-heptane extract of *Cassia fistula* Linn. leaves was shown to protect the liver against CCl₄ induced liver damage as indicated by significant reversal of the increase in the activities of transaminases, bilirubin and ALP in serum (Bhakta et al., 1999). Further, this extract was also shown to protect the liver against paracetamol induced hepatocellular damage (Bhakta et al., 2001).

These reports formed the basis to conduct experiments in this laboratory to investigate the hepatoprotective and antioxidant properties of the ethanolic leaf extract of *Cassia fistula* Linn. on its pre-treatment against CCl₄ induced liver damage. Administration of 500 mg/kg of the ethanolic leaf extract, significantly decreased the increase in the activities of AST, ALT, ALP and bilirubin in serum. Further, the leaf extract also prevented the five fold increase in the levels of LPO and reversed the decrease in the activities of GR and CAT induced by CCl₄ treatment towards normalcy in the liver tissue.
of rats, suggesting both the hepatoprotective and antioxidant properties of the leaf extract during its pre-treatment against CCl₄ induced hepatotoxicity (Pradeep et al., 2005). Moreover, post treatment as well as simultaneous administration of ethanolic leaf extract of Cassia fistula Linn. has demonstrated significant hepatoprotective and antioxidant activities against CCl₄ (VictorRajMohan et al., 2003a,b) and Isoniazid (VictorRajMohan et al., 2004) induced hepatocellular damage in rats. Additionally, post treatment of ethanolic leaf extract of Cassia fistula Linn. also demonstrated significant protection against diethylnitrosamine induced latent hepatotoxicity in rats (Pradeep et al., 2004). These observations clearly demonstrate the hepatoprotective and antioxidant properties of leaf extract of Cassia fistula Linn. against xenobiotics and drug induced hepatotoxicity.

In view of the above findings, it was considered of interest to investigate the hepatoprotective, antioxidant and antilipidemic properties of the ethanolic leaf extract of Cassia fistula Linn. during its simultaneous treatment against CCl₄, INH alone and antitubercular drugs (INH+RIF+PYR) induced hepatotoxicity using rats as animal models.

1.12 Silymarin

Silymarin (SIL), a flavonolignan which is extracted from the seeds and fruits of the plant Silybum marianum, belongs to the family Compositae. The main flavanolignans in SIL are Silibin (also known as Silibinin), Silydianin, Isosilybin and Silychristin. Silibin, which constitutes 50 to 70% of SIL is regarded as the single most important component of SIL. Extracts obtained
from *Silybum marianum* (also called “Milk Thistle”) was used even from 4th century BC for the treatment of plague and congestive conditions of the liver and spleen (Choksi *et al*., 2000). SIL is recently used as hepatoprotective agent for numerous liver disorders characterized by degenerative necrosis and impairment in the function of the liver. In European countries, SIL is widely used for protection against various hepatobiliary problems such as hepatitis, cirrhosis, gallstones and jaundice (Flora *et al*., 1998). The German Commission E recommends it for the treatment of dyspeptic complaints, toxin-induced liver damage, liver cirrhosis as well as supportive therapy for chronic liver inflammatory conditions (Belmenthal, 1998). It also offers protection against chemical hepatotoxins such as CCl₄ (Muriel and Mourelle, 1990), acetaminophen (Muriel *et al*., 1992), alcoholic liver diseases (Feher *et al*., 1989), phalloidin, galactosamine and thioacetamide (Fraschini *et al*., 2002).

The ability of SIL to protect the hepatocyte membrane against toxicity induced by xenobiotics is well documented. SIL has been shown to exhibit membrane stabilizing action by virtue of its antioxidant and free radical scavenging properties (Valenzuela and Garrido, 1994; Morazzoni and Bombardelli, 1995; Basaga *et al*., 1997). Moreover, SIL has been shown to enhance endogenous antioxidant defence such as reduced glutathione. Another interesting property of SIL and Silibin is their role in the regulation of content of GSH in various organs (Valenzuela *et al*., 1989). Due to its hepatoprotective and antioxidant properties, SIL has been recommended for use as an adjunct in the treatment of liver diseases rather successfully in liver
cirrhosis, although its clinical efficacy is continuously discussed (Flora et al., 1998; Saller et al., 2001). SIL has been shown to inhibit cytochrome P450 systems (phase I metabolism) and other hepatic cytochrome enzyme activities (Baer-Dubowska et al., 1998). Further, this flavonoid has been shown to effectively scavenge free radicals, prevent lipid peroxidation and inhibit cytochrome P450 detoxifying systems to protect the liver against hepatotoxins such as ethanol (Fraschini et al., 2002). Due to the above specified hepatoprotective and antioxidant properties, SIL is preferred as a standard hepatoprotective agent against experimentally induced hepatotoxicity in animal models. However, studies regarding the potency of SIL against INH and/or its combination with other antitubercular drugs induced hepatotoxicity is unavailable. Moreover, its efficacy against INH and antitubercular drugs induced hepatic steatosis has not been evaluated. Hence, in this study, it was considered of interest to evaluate the efficacy of SIL for its hepatoprotective, antioxidant and antilipidemic properties against INH and antitubercular drugs induced hepatotoxicity, which is manifested as either hepatocellular necrosis or fatty infiltration or a combination of both.

1.13 Lacunae

Several principles extracted from plants have been shown to protect the liver against antitubercular drugs induced hepatotoxicity. However, the hepatoprotective, antioxidant and antilipidemic properties of ethanolic leaf extract of Cassia fistula Linn. and the plant flavonoid Silymarin against INH alone or its combination with other antitubercular drugs induced hepatotoxicity remains unexplored. Hence, this study was conducted to
evaluate the hepatoprotective, antioxidant and antilipidemic properties of ethanolic leaf extract of *Cassia fistula* Linn. and to compare its efficacy with Silymarin against INH alone or in its combination with antitubercular drugs (INH+RIF+PYR) induced hepatocellular necrosis and hepatic steatosis.

1.14 Hypothesis

The active principles extracted from plants offer protection against drugs and chemicals induced hepatotoxicity. It is likely that the ethanolic leaf extract of *Cassia fistula* Linn. (which contains flavonoids, glycosides, alkaloids and tannins as active principles) and Silymarin (rich in flavonolignans) would offer hepatoprotective and antioxidant properties on their simultaneous treatment against CCl₄, INH alone or in its combination with antitubercular drugs (INH+RIF+PYR) induced hepatotoxicity.

1.15 Objectives

The present study was designed to evaluate the hepatoprotective and antioxidant properties of ethanolic leaf extract of *Cassia fistula* Linn. and SIL against CCl₄, INH and antitubercular drugs (INH+RIF+PYR) induced hepatotoxicity. The objectives of the present study are as follows:

- To induce liver toxicity using CCl₄ and to evaluate the hepatoprotective and antioxidant property of ethanolic leaf extract of *Cassia fistula* Linn. during its simultaneous treatment.
To induce liver toxicity by administration of INH and to evaluate the hepatoprotective, antioxidant and antilipidemic properties of ethanolic leaf extract of *Cassia fistula* Linn. and Silymarin during its simultaneous treatment with INH.

To induce liver toxicity by combined administration of INH, RIF and PYR and to evaluate the hepatoprotective, antioxidant and antilipidemic properties of ethanolic leaf extract of *Cassia fistula* Linn. and Silymarin during its simultaneous treatment with INH, RIF and PYR.

To investigate the histopathological changes, if any, upon administration of CCl₄, INH alone, INH+RIF+PYR, ethanolic leaf extract of *Cassia fistula* Linn. and Silymarin.

The experimental models adopted to fulfill the objectives, parameters investigated, results obtained, relevant discussions thereof and eventual inferences drawn are embodied in the forthcoming parts of this thesis.