AIMS & OBJECTIVES
REVIEW
OF
LITERATURE
By 1970’s Ethambutol, Rifampicin (RMP) and Pyrazinamide were also added in the chemotherapy of Tuberculosis, and the conventional 12-18 month treatment was largely replaced by more effective and less toxic 6-9 month short-course chemotherapy (Davis AL 2000).

From 1980 onwards, short-course Chemotherapy was applied nationwide and comprised of 6 months of daily doses of INH and RMP, supplemented with Pyrazinamide, and either Streptomycin, or Ethambutol during the first 2 months (Chaulet P et al 2000).

Isoniazid, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin are the five first line drugs against Tuberculosis. Out of these INH and RMP are the two most active drugs. INH-RMP combination administered for 9 months cures 95-98% of cases of Tuberculosis by susceptible strains (Chambers HF 2004). Unfortunately, both these drugs have an associated potential of causing significant hepatotoxicity. Transient increases in hepatic transaminases are also common, but no action is required unless the patient has symptoms of hepatitis. When clinically evident hepatitis with anorexia, nausea, vomiting, jaundice, hepatic enlargement & tenderness occurs, the drugs have to be curtailed, else it can be fatal (Girling DJ 1982).

ISONIAZID

Isoniazid also referred to as INH (isonicotinic acid hydrazide), is a nearly perfect chemotherapeutic agent for the treatment of
Tuberculosis. It is bactericidal, inexpensive, and well absorbed when administered orally or parenterally. It is an essential component of all antitubercular regimens, rapidly kills fast multiplying organisms both intracellularly and extracellularly, but is bacteriostatic for the resting bacilli (Tripathi KD 2003).

Mechanism of Action

The probable mechanism of its action is that it inhibits the mycobacterial desaturase that catalyses the first reaction specific to mycolic acid synthesis. Mycolic acids are unique components of mycobacterial cell walls and this would explain the high degree of selectivity of the antimicrobial activity of the drug (Tripathi KD 2003).
Pharmacokinetics

INH is rapidly and completely absorbed orally or parenterally and produces peak blood levels within 1 to 2 hours. It readily penetrates all body tissues, tubercular cavities, placenta and meninges.

The half-life of INH is widely variable and dependent on acetylator status. It is extensively metabolized in liver; most important pathway being acetylation. Fast acetylators metabolize the drug about 5 to 6 times faster; however, the rate of acetylation dose not
significantly alter the effectiveness of INH. The unchanged drug and the metabolites are excreted in urine (Riley MR et al 2001).

In recent years, Isoniazid has been used not only to treat patients with active tuberculosis but also with positive tuberculin reactivity, (Comstock GW et al 1972) and those at high risk of developing active Tuberculosis (Horwitz O et al 1966).

The widened scope of Isoniazid administration was predicated largely on the belief that the drug caused no serious side effects.

**Adverse effects**

Isoniazid is well tolerated by most patients. Peripheral neuritis and a variety of neurological manifestation are important dose-dependent toxic effects in frequently seen with the standard dose (at 3-5 mg/kg/day, the incidence is only 2%). Isoniazid induced hepatitis is the most frequent major toxic effect (mild and transient elevation of serum transaminases is seen in 10-20% of patients). Fever and skin rashes (1%-2%) are occasionally seen (Riley MR et al 2001).

**Isoniazid induced Hepatotoxicity**

Isoniazid has been used clinically since 1952, and although the sporadic case reports of suspected INH induced hepatotoxicity surfaced shortly after the release of INH, its potential to produce hepatotoxicity was not fully appreciated for almost 20 year (Sarich TC et al 1995). In 1969, Scharer and Smith reported that 10.3% of patients receiving Isoniazid developed liver function abnormalities (Scharer L et al 1969). Subsequently in a large study of 2321 patient
who were of Isoniazid Prophylaxis, Clinical Hepatitis was reported to occur in 19 (0.8%) cases and overt Jaundice in 13 (0.6%) cases with one death (Garibaldi RA et al 1972). In a randomized, double blind study the relative risk of developing hepatotoxicity due to Isoniazid Chemoprophylaxis for less than one year was found to be 5.2/1000 irrespective of age (Riska N 1976).

It is reported that daily INH administration is associated with mild elevation of liver enzyme activities in plasma in upto 20% of patients, and significant hepatotoxicity (predominantly hepatic necrosis) in approximately 1-2% of patients.

**RIFAMPICIN**

Rifampicin (RMP) is a semisynthetic derivative of rifamycin which is produced by *Streptomyces mediterranei*. It is bactericidal to *Mycobacterium Tuberculosis* and is as efficacious as INH for the treatment of Tuberculosis. It acts best on slowly or intermittently dividing bacilli. Both extracellular and intracellular bacilli are affected. It has good sterilizing and resistance preventing actions (Tripathi KD 2003).

**Mechanism of Action**

The exact mechanism of Rifampicin-induced Hepato and Renal toxicity is unknown. The predominant pathway of Rifampicin Metabolism is desacetylation into desacetyl-Rifampicin and hydrolysis produces a 3-formyl Rifampicin (Holdiness MR 1984; Acouella G et al 1980). Rifampicin is a potent inducer of hepatic CYP450 System in
the liver and intestine, there by increasing metabolism of many other compounds (Kolars JC et al 1992; Combalbert J et al 1989).

Rifampicin acts by inhibiting bacterial DNA-dependent RNA polymerase and thereby inhibits DNA-dependent RNA synthesis (Tripathi KD 2003).

Pharmacokinetics

It is well absorbed orally and achieves mean peak plasma levels within 1-4 hours. It is widely distributed in the body and penetrated in many body tissues, including the cerebrospinal fluid. It is metabolized in the liver by deacetylation, excreted mainly in bile and undergoes enterohepatic circulation. The half life is approximately 3 hour after 600 mg oral dose. Rifampicin is as enzyme inducer (of most Cytochrome-P450 isoforms) and when given with Isoniazid induces its metabolism (Riley MR et al 2001).

Adverse effects

Rifampicin generally is well tolerated. It imparts a harmless orange colour to all body secretion. Occasional adverse effects include rashes (1% to 5%), fever (0.5%), epigastric distress, nausea, vomiting (1% to 2%) thrombocytopenia, and nephritis (rarely). It may cause cholestatic jaundice and occasionally hepatitis (<1%) (Riley MR et al 2001).

Rifampicin induced Hepatotoxicity

Rifampicin when given alone rarely causes hepatitis (Steele MA et al 1991; Capelle P et al 1972). It inhibits both uptake and
excretion of bilirubin in a dose related manner giving rise to elevated plasma levels of conjugated and unconjugated bilirubin without producing parenchymal injury (Capelle P et al 1972). Rifampicin appears to interfere mainly with the uptake of bilirubin by effect of the membrane receptor protein of the hepatocyte, leading to elevated levels of unconjugated protein. Competitive interference with excretion of conjugated bilirubin has also been demonstrated (Capelle P et al 1972).

PYRAZINAMIDE

The exact mechanism of action by which Pyrazinamide inhibits the growth of M. Tuberculosis organisms is unknown. In vitro and in vivo studies have demonstrated that Pyrazinamide is only active at a slightly acidic pH (pH 5.5) (Steelae MA et al 1988; Mandell GL et al 1990; Girling DJ 1984). Pyrazinamide is well absorbed from the gastrointestinal tract (Rifater 2000; Pyrazinamide (Lederle) 2000).

Mechanism of Action

Pyrazinamide is hydrolyzed by a microsomal deamidase to pyrazinoic acid, an active metabolite, and then hydroxylated by xanthine oxidase to 5-hydroxypyrazinoic acid (Steel MA et al 1988; Lacroix C et al 1989; Stamatakis G et al 1988).
Pharmacokinetics

Pyrazinamide is widely distributed to most fluids and tissues, including liver, lungs, kidneys, and bile. Pyrazinamide has excellent penetration into CSF, ranging from 87 to 105% of the corresponding serum concentration (Ellard GA 1987; Phuapradit P et al 1990).

ETHAMBUTOL

Molecular weight of Ethambutol is 277.23. Ethambutol is rapidly absorbed from the gastrointestinal tract following oral administration.

Mechanism of Action

Ethambutol diffuses into actively growing *M. Tuberculosis* such as tubercle bacilli. Ethambutol appears to inhibit the synthesis of one
or more metabolites, thus causing impairment of cell metabolism, arrest of multiplication, and cell death. No cross resistance with other available antimicrobial agents has been demonstrated (Myambutol (Lederle) 2000).

**Pharmacokinetics**

Ethambutol is distributed to most tissues and body fluids, except CSF. Ethambutol does not penetrate intact meninges, but 10 to 50% may penetrate the meninges of patients with tuberculous meningitis (Myambutol (Lederle) 2000).

**ALLOPURINOL**

**Incidence of Gout**

Gout is a disease characterized biochemically by hyperuricemia and clinically by episodes of severe, acute arthritis. When not treated, it passes through four stages: asymptomatic hyperuricemia, acute Gouty arthritis, intercritical (interval) period, and chronic tophaceous Gout.

Hyperuricemia may be (a) primary, when it results from a metabolic error that leads to an overproduction and/or decreased clearance of uric acid; or (b) secondary, when it is a consequence of another disease or of drug therapy (Andrejus Korolkovas 1988).

Gout is more common in older individuals, with the first Gout attack usually occurring between the ages of 40-60. Women rarely develop Gout until after menopause. Only 10-20% of patients with Gout have a family history of the disorder.
Definition of Gout

**Gout** is a metabolic disease in which increased levels of uric acid in the blood become deposited within joints and tissues. The major cause of Gout is the ineffective metabolism of a nucleic acid in the body called purine. The normal metabolism of purine results in an end product called uric acid, which then gets excreted by the kidneys. In Gout, however, metabolic problems cause uric acid to be overproduced and/or under excreted. In about 90% of cases, Gout is due to the under excretion of uric acid from the kidneys. When uric acid builds up in the blood, it causes the formation of needle-like crystals (called urate crystals) which irritate the joint lining and cause severe joint inflammation, or an acute Gout attack. The urate crystals also may form a deposit in the joint (called a tophus). In about 3 out 4 people with Gout, the big toe joint is affected, but any joint may be involved including the knees, ankles, fingers, wrists, or elbows. Women, in particular, are likely to experience Gout in the hands. Another possible result of high blood uric acid levels is the development of kidney stones.

It is very likely that one of the first useful drugs in Gout was an extract from the plant *Colchicum autumnale*. Preparations from this plant have been used since the sixth century. Colchicum was known as *hermodactyl* (finger of Hermes) and *articulorum* (soul of the joints), because of its effect in the relief of pain of articular origin. In 1763, **von Storck** introduced it for the treatment of acute Gout. In 1820,
Pelletier and Caventou isolated from the plant the alkaloid colchicine. Its chemical structure was determined by Dewar in 1945. Its total synthesis was performed independently by Eschenmoser and van Tamelen and co-workers in 1959.

Clinical studies with anti-inflammatory drugs, such as adrenal corticosteroids, corticotropin, 3, 5-pyrazolidinediones, and arylalkanoic acids, led to their introduction in the therapy of acute Gouty arthritis.

Probenecid was first synthesized by Miller in 1950 and evaluated by Beyer et al. that same year. Structurally, it is related to carinamide, a compound with uricosuric activity and then used to increase penicillin blood levels. Probenecid was therefore designed as a potential depressor of renal tubular excretion of penicillin blood levels. Probenecid was therefore designed as a potential depressor of renal tubular excretion of penicillin at a time when this antibiotic was scarce.

Allopurinol is a product of rational drug design. From 1942 on, Hitchings, Elion and coworkers, in an effort to introduce new antineoplastic agents, started to synthesize and evaluate potential antimetabolites of the purine and pyrimidine bases of the nucleic acids. Allopurinol was designed to be an inhibitor of xanthine oxidase, the enzyme that catalyzes the biosynthesis of uric acid. Tried first in leukemic patients, it produced a marked decrease in plasma and urinary levels of uric acid. This effect suggested its potential
usefulness in the therapy of primary Gout. In 1963, Rundles and co-workers performed, with great success, the first clinical trial of Allopurinol in Gout. Later it was seen that its metabolite, oxypurinol, is equally active.

Allopurinol (C₉H₆N₄O) is used to treat hyperuricaemia associated with a variety of conditions including chronic Gout. Common adverse effects include skin rashes and hypersensitivity reactions; acute attacks of Gout may initially be precipitated.

General Properties

A white or off-white, almost odourless powder. The melting point is above 350°C. The molecular weight is 136.11. C = 44.12%, H = 2.96%, N = 41.16% and O = 111.75%. It is a tautomeric mixture of 1H-pyrazolo [3,4-d] pyrimidin-4-ol and 1,5-dihydro-4H-pyrazolo[3,4-d] pyrimidin-4-one. Very slightly soluble in water and alcohol; practically insoluble in chloroform and in ether; dissolves in dilute solutions of alkali hydroxides.

Xanthine oxidase Allopurinol for Gout

Xanthine oxidase is the last enzyme on the breakdown pathway of purine bases in primates and it catalyses the conversion of hypoxanthine to xanthine and of xanthine to uric acid. The latter is normally excreted, although quantities of the other purines may also
find their way into the urine. In some diseases, notably Gout, the production of purines can be increased as a primary cause of the disease. Enzyme deficiencies with a genetic origin may play a part. One such case is deficiency of the salvage enzyme hypoxanthine phosphoribosyltransferase (HPRT) which leads to an elevated level of hypoxanthine phosphoribosylpyrophosphate. The latter stimulates de novo purine biosynthesis at the initial rate-limiting step of the formation of phosphoribosylamine.

The consequence of increased purine synthesis is an increased throughput down the catabolic pathway to uric acid. When levels of the latter rise above saturation, crystals of monosodium urate form in the synovial fluid. The characteristic symptoms of Gout derive from an inflammatory response to these crystals and thus closely resemble the painful joint swellings in rheumatoid arthritis. This may occur in one joint only or in several. In advanced Gout, deposits (tophi) of sodium urate form on or near joints or tendon sheaths, which are soft initially but eventually harden.

For therapy the major need is to lower serum uric acid levels, although anti-inflammatory drugs will relieve the symptoms on a short-term basis. One of the most useful drugs in effecting a long-term cure is Allopurinol. Xanthine oxidase is the target of the drug, and therefore serum and urine hypoxanthine and xanthine levels are raised while, more importantly, uric acid levels are lowered. In addition, the drug is useful when given in combination with anti-


Some drugs since serum urate levels can rise sharply as the 
erythema cells die. This is an example of secondary Gout, secondary 
uric acid formation is increased as a consequence of other 
metabolic disturbances. In this case, the danger is not only that acute episodes of 
may occur, but also that sodium urate crystals may form in the 
distal tubule of the kidney.

Clearly, if a drug is metabolised by xanthine oxidase, its action 
is likely to be potentiated by Allopurinol. For example, 6-
mercaptopurine (a drug used for the treatment of leukaemia) is 
metabolized by xanthine oxidase to 6-thiouric acid, an inactive 
metabolite. The dose of mercaptopurine required when given in 
conjunction with Allopurinol must therefore be reduced to avoid 
widespread toxicity which would otherwise occur if higher 
mercaptopurine levels were sustained for longer periods of time.

Xanthine oxidase is a complex enzyme containing, in effect, a 
transport system involving molybdenum, flavin nucleotide and two 
iron-sulphur centres which convey electrons to oxygen to yield 
superoxide ion (O₂⁻). Allopurinol inhibits the enzyme in a complex 
fashion, and may be regarded as one of the earliest examples of a 
suicide substrate. If the inhibition is studied without pre-incubation of 
enzyme and inhibitor, Allopurinol behaves as though it were as 
competitive inhibitor with a Kᵢ of 7×10⁻⁷ M. With pre-incubation in the 
presence of air, the inhibition increases and it is no longer competitive 
with substrate. Allopurinol is also a substrate for xanthine oxidase.
and the product of the reaction, oxypurinol (alloxanthine), is also an inhibitor. In the presence of xanthine as substrate and oxygen, or anaerobically without substrate, the enzyme is inactivated by oxypurinol. If the oxidation of xanthine, which require the enzyme to cycle between reduced and oxidized forms, and for the enzyme to be in an anaerobic environment, both result in enzyme inactivation by oxypurinol, it is likely that the reduced form of the enzyme is sensitive to oxypurinol. The dissociation constant of the oxypurinol-enzyme complex is $5.4 \times 10^{-10}$ M. Inhibition can be reversed by prolonged dialysis or by allowing the complex to be reoxidized in the presence of air, thus confirming that it is the partly reduced form of the enzyme that is receptive to oxypurinol inhibition.

The inactivation of reduced xanthine oxidase by oxypurinol follows first-order kinetics by appearing to be dependent on the concentration of reduced enzyme. This may be the result of an internal rearrangement of the enzyme-inhibitor complex in a time-dependent fashion. The similarity between the tight or stoichiometric binding of oxypurinol to xanthine oxidase, and of coformycin to adenosine deaminase was noted by Cha et al (1975).

Allopurinol has been found to be effective in the treatment of kala-azar (leishmaniasis). In this instance the drug is acting as a false substrate for the parasites hypoxanthine phosphoribosyltransferase much more efficiently binds for the human erythrocyte enzyme. Subsequent enzymes convert the ribonucleotide into an analogue of
ATP which is then incorporated into a faulty RNA (Christopher J. Coulson 1994).

Mechanism of Action

The primary event in acute Gouty arthritis is the local deposition of crystalline monosodium urate hydrate. Ingestion of the crystals by neutrophilic leukocytes leads to activation and release of lysosomal enzymes. The negatively charged urate crystals also activate complement and Hageman factor. The latter initiates the clotting mechanism and the kinin cascade resulting in pain, increases of vascular permeability, and accumulation of leukocytes.

Uricosurics, such as probenecid and sulfinpyrazone, promote the excretion of uric acid by inhibiting the tubular reabsorption of filtered urate and thereby lower the urate level in the blood. In consequence of this action, tophi formation is decreased or prevented, existing urate deposits are resolved, and after several months of treatment, the frequency of acute attacks of Gout is reduced.

Allopurinol, as well as its metabolic product oxypurinol, reduce the biosynthesis of uric acid from xanthine. They act as inhibitors of xanthine oxidase, the enzyme that converts hypoxanthine to xanthine and xanthine to uric acid. Allopurinol binds 15 times more tightly to xanthine oxidase than its own natural substrate, xanthine. It inhibits also de novo purine synthesis through a feedback mechanism in thoses patients who possess the enzyme hypoxanthine-guanine phosphoribosyltransferase. By decreasing both serum and urine
concentrations of uric acid, Allopurinol and related compounds prevent or lower urate deposition and thereby hinder then occurrence or progression of both urate nephropathy and Gouty arthritis. Patients with chronic Gout may have prevented or decreased tophi formation and chronic joint changes, resolved existing urate crystals and deposits, and after several months of treatment, reduced the frequency of acute attacks of Gout.

**Adverse Effects**

The most common side-effect of Allopurinol is skin rash. Rashes are generally maculopapular or pruritic, but more serious hypersensitivity reactions may occur and include exfoliative rashes, the Stevens Johnson syndrome, and toxic epidernal necrolysis. It is therefore recommended that Allopurinol be withdrawn immediately if a rash occurs. Further symptoms of hypersensitivity include fever, chills, leucopenia or leucocytosis, eosinophilia, arthralgia, and casculitis leading to renal and hepatic damage. These hypersensitivity reactions may be severe, even fatal, and patients with hepatic or renal impairment are at special risk.

Hepatotoxicity and signs of altered liver function may be found in patients not exhibiting hypersensitivity.

Many other side-effects, usually of a less serious nature, have been noted and include peripheral neuritis, alopecia, hypertension, taste disturbances, nausea, vomiting, abdominal pain, diarrhoea, headache, drowsiness, and vertigo.
In addition to these adverse effects patients may experience an increase in acute Gouty attacks during the first few months of treatment.

A Boston Collaborative Drug Surveillance Program of 29524 hospitalised patients revealed that, with the exception of skin reactions, of 1835 patients treated with Allopurinol 33 (1.8%) experienced adverse effects. It appeared that although Allopurinol is seldom associated with toxicity, when it does occur it can be of a serious nature. Adverse effects were dose-related and the most frequent were haematological (11 patients, 0.6%), diarrhoea (5 patients, 0.3%), and drug fever (5 patients, 0.3%). Hepatotoxicity was reported in 3 patients (0.2%). Two patients developed possible hypersensitivity reactions to Allopurinol.

Another analysis involving 1748 outpatients indicated no instances of acute blood disorders, skin diseases, or hypersensitivity that warranted hospital treatment. Liver disease, although found, was considered to be unassociated with Allopurinol. There were only 2 patients in whom renal disease could possibly have been caused by Allopurinol.

In addition to the haematological abnormalities of leucopenia, thrombocytopenia, haemolytic anaemia, and clotting abnormalities noted in the Boston Collaborative Drug Surveillance Program, aplastic anaemia has also been reported, sometimes in patients with impaired renal function.
HEPATOTOXICITY DUE TO TREATMENT WITH ISONIAZID AND RIFAMPICIN COMBINATION

There is evidence to suggest that drug induced hepatitis occurs with greater frequency and may be more severe when Isoniazid and Rifampicin are given in combination than when either drug is given alone (Lal S et al 1972; Snider et al 1984). Few reports have suggested that hepatitis appeared sooner with Isoniazid and Rifampicin combination than with Isoniazid therapy alone, with significant elevation of transaminase levels (Tsagaropoulou SH et al 1985; Pessayre D et al 1977).

In a meta-analysis study it was reported that the incidence of clinical hepatitis in adults with Isoniazid alone was 0.6%, with multidrug Isoniazid regimens without Rifampicin, 1.6%, and with regimens containing Rifampicin and not Isoniazid, 1.1%. However the incidence in patients taking both Isoniazid and Rifampicin was 2.6%, which was significantly higher in comparison (Garibaldi RA et al 1972).

Experimental studies also have shown that administration of both drugs concurrently is more hepatotoxic than of either drug alone (Kalra BS et al 2004).

Patients at high risk of developing hepatotoxicity to anti-tubercular drugs include patients with suspected liver disease, alcoholics, malnourished patients and possibly the elderly and children (Garg PK et al 2001).
Mechanism of Hepatotoxicity

The exact mechanism responsible for injury caused by INH-RMP combination to hepatocytes is still controversial. Since no correlation was found between Isoniazid plasma concentrations and susceptibility to Isoniazid liver injury, it was thought that a toxic metabolite rather then the drug itself might be responsible for Isoniazid induced hepatitis (Mitchell JR et al 1976). Presently, Isoniazid metabolites, acetylhydrazine and hydrazine, have each been implicated as the causative hepatotoxin.

Isoniazid is metabolized in the liver by acetylation, hydrolysis and by oxidation. Acetylation is quantitatively the most important step in the pathway. Isoniazid is acetylated by N-acetyl transferase to acetylIsoniazid, which is hydrolysed to isonicotinic acid and acetylhydrazine. Acetylhydrazine is further acetylated to non toxic diacetylhydrazine and may be converted by the hepatic microsomal enzyme (CytP450) to the reactive metabolite which causes liver necrosis in animals (Thompson NP et al 1995, Sarma GR et al 1986). Rifampicin, a powerful inducer of Cyt P450 contributes to the hepatotoxicity by increasing the fraction of a dose of INH metabolized through the toxifying pathway (Steele MA et al 1991; Piriou et al 1983; Capelle P et al 1972). It has been observed that slow acetylators have an increased susceptibility to the hepatotoxic effects of Isoniazid. Although slow acetylators generate less acetylhydrazine from a given dose of Isoniazid, the rate of acetylation of
acetylhydrazine to non toxic diacetylhydrazine is so much slower that more acetylhydrazine will be available to the toxifying pathway (Lauterburg BH et al 1985).

Hydrazine is formed directly by hydrolysis of INH as well as from acetylhydrazine by the action of an enzyme Amidohydrolase/Amidase. Hydrazine, like acetylhydrazine, gets metabolized by Cytochrome P450 (Cyt P450) to reactive intermediates (Sarma GR et al 1986, Noda A et al 1985, Jenner AM et al 1994). Thus Cyt P450 enzymes are critical in hepatotoxicity in that they lead to reactive, toxic metabolites.

The chemically reactive metabolites of acetylhydrazine and hydrazine, thus generated by Cyt P450 covalently bind to microsomal proteins, causing injury to the macromolecules of hepatocytes (Timbrell JA et al 1980; Sarma GR et al 1986).

It is becoming increasingly apparent that many reactive intermediates formed during the INH metabolism are free radicals (Timbrell JA et al 1980, Noda A et al 1985, Augusto O et al 1985, Trush MA et al 1982). Free radicals are chemical species possessing an unpaired electron which are generally very reactive. The generation of free radicals, presents a danger to cells because radicals are capable of interacting with, and subsequently damaging, the entire array of biomolecules which constitute cells. The radical-initiated processes are particularly deleterious because they are conservative and propagative. To circumvent the damages caused by free radicals,
multiple defense systems, collectively called antioxidants (Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-PX), Glutathione (GSH), Tocopherol, etc), are present in living organisms (Mates JM et al 1999). For a free radical intermediate to initiate membrane lipid peroxidation, antioxidant levels would have to be decreased significantly. This is supported by the fact that microsomes isolated from tocopherol-deficient animals are much more susceptible to radical-initiated lipid peroxidation (Trush MA et al 1982). The generation of free radicals overwhelms the antioxidant defenses in the liver and results in oxidative destruction of cellular membranes and serious tissue damage (Cheeseman KH et al 1993). Other examples of toxic compounds exerting their toxicity via the production of free radicals are now well established. The classic example is carbon tetrachloride, which gets metabolized to the trichloromethyl free radical by the action of Cyt P450 in the liver (Slater TF 1966; Cheeseman KH et al 1993).

Recent studies have suggested that superoxide is involved in INH activation, and Reactive Oxygen Species (ROS) such as NO, O₂⁻, H₂O₂, and OH, arise during this activation (Albano E et al 1987, McCord JM 1983, Johansson K et al 1995, Wang JY et al 1998).

The role of oxidative stress as a mechanism of hepatotoxicity caused by INH and RMP has been investigated in young growing rats (Sodhi CP et al 1997). A successful model of hepatotoxicity was produced by giving 50 mg/kg/day each of INH and RMP
intraperitoneally to rats for two weeks. Analysis of serum transaminases and histopathological observations revealed presence presence of hepatic injury. The Glutathione and related thiols were significantly decreased in both blood and liver tissues. The decrease in Glutathione, coupled with enhanced endogenous radical generation, leads to build up of free radicals. This build up has an inhibitory effect on the protective enzymes. The altered profile of antioxidant enzymes along with increased lipid peroxidation indicated the enhanced oxidative stress in treatment with INH and RMP combination (Sodhi CP et al 1997).

Some in vitro studies had also demonstrated that the INH metabolites bind with glutathione resulting in its depletion (Nelson SD et al 1976). Other studies, relating suppression of the antioxidant system with antiTuberculosis drugs in rats, have also been reported (Skakun NP et al 1992).

The current data suggests the potential role of Cytochrome P450 inhibitors and antioxidants in the prevention of hepatotoxicity due to antitubercular drugs.

LIVER ENZYMES

Elevations of liver enzyme activity in plasma are associated with acute hepatocellular necrosis and reflect the release of enzymes from the cytoplasm of dying cells. Increased activity of liver cell enzymes is thought to be a more sensitive indicator of cellular damage than are most other biochemical indices and most morphological findings.
Serum transaminases that are commonly employed as liver function tests are Alkaline Phosphatase (ALP) and Alanine transaminase (ALT).

Alkaline Phosphatase (ALP) is a mitochondrial enzyme present in large quantities in liver, heart, skeletal muscle and kidney. The serum levels increase whenever these tissues are acutely damaged and therefore is a note a specific indicator of liver injury. Its normal value in humans is 0-10 U/Lit.

Alanine transaminase (ALT or SGPT) is an ecto-enzyme, localised in cell membranes. High levels of ALP is noticed in extrahepatic obstruction. Intrahepatic cholestasis may be due to virus or by drugs (chlorpromazine). ALP is produced by epithelial cells of biliary canaliculi and obstruction of bile with consequent irritation of epithelial cells leads to Secretion ALP into serum. Its normal value in humans is 40-125 U/Lit. Enzyme level increasing by three folds from the baseline is considered significant.

Baleza et al assessed ALT as liver function test in rats subjected to treatment with various hepatotoxic including ethione, carbon tetrachloride, allyl alcohol and thioacetamide. The agents associated with severe necrotic produced pronounced elevations of ALT. Since ALT activity resides primarily in the liver, its estimation is a more reliable parameter for evaluating hepatic injury in animals.

**POTENTIAL HEPATOPROTECTIVE AGENTS**

Treating Tuberculosis in patients with antituberculosis-treatment induced hepatotoxicity poses a difficult clinical challenge. It
is essential to first stop all potentially hepatotoxic drugs till complete clinical and biochemical resolution of hepatitis is there. In the interim period, non hepatotoxic drugs such as Ethambutol, Streptomycin and quinolones (second line drugs for treatment of Tuberculosis) such as ofloxacin or ciprofloxacin can be started. However the second line drugs are not as efficacious as the first line drugs, and are toxic on long term therapy. This may further compromise the health of the patient and consequent clinical outcome, further exposing community to inadequately treated infected patient (Thompson NP et al 1995).

An alternative to overcome the problem of hepatotoxicity is to use the hepatoprotective agents along with the first line drugs as a primary preventive measure. At present there are no such hepatoprotective agents in clinical practice. However a few including bis-p-nitrophenyl phosphate (Sarich TC et al 1999), 1-thyroxine (Sarich T C et al 1998), roboxin (Skakun NP et al 1991), N-acetylcysteine (Attri S et al 2001), cimetidine (Lauterburg BH et al 1985) and α-Tocopherol (Skakun NP et al 1991) are being evaluated in animal models of antitubercular drug induced hepatotoxicity.

There is paucity of literature on the hepatoprotective potential of cimetidine in INH-RMP induced hepatotoxicity. However Karla BS, Gupta U studied the role of cimetidine as a hepatoprotective agent against INH-RMP induced hepatotoxicity in albino rabbits and have observed promising results (Kaira BS et al 2004).

Vitamin E, first discovered in 1922, is known to be a mixture of
four tocopherols (alpha, beta, delta, and gamma). The alpha fraction is the most often used in antioxidant of the four naturally occurring tocopherols and is most often used in experiments involving Vitamin E as an antioxidant (Brent JA et al 1993; Marchlin et al 1984).

Alpha tocopherol is described as a chain breaking antioxidant because of its ability to transfer a phenolic hydrogen to peroxyl free radical (LOO') of peroxidized polyunsaturated fatty acids, thereby interfering with chain propagating step of lipid peroxidation (Brent JA et al 1993, Marchlin et al 1984).

\[ \text{LOO}^\prime + \alpha - \text{tocopherol} - \text{OH} \rightarrow \text{LOOH} + \alpha - \text{tocopherol} - \text{O}^\prime \]

Once formed, the alpha tocopherol radical (alpha tocopherol-O') is relatively stable and, in normal circumstances, insufficiently reactive to initiate lipid peroxidation itself, an essential criterion of a good antioxidant (Chesseman KH et al 1993). It may be reduced by Glutathione or it may combine with another radical to form a dimer (Comporite M 1985).

\[ 2 (\alpha - \text{tocopherol} - \text{O}^\prime) + 2 \text{GSH} \rightarrow \alpha - \text{tocopherol} - \text{tocopherol} - \alpha + \text{GS-SG} \]

\[ 2 (\alpha - \text{tocopherol} - \text{O}^\prime) \rightarrow \alpha - \text{tocopherol} - \text{tocopherol} - \alpha \]

Studies have shown that treatment with antioxidants (LIV.100) offers protection, against Isoniazid-induced hepatotoxicity in rats, by reducing lipid peroxidation and restoring the antioxidant defense system (Saraswathy SD et al 1998).
8D et al 1998]. Novel compounds like Isonicotinoylhydrazones, which are structurally related to Isoniazid, have been designed and show to protect against oxidative hepatic injury of Isoniazid because of their superoxide scavenging (antioxidant) action (Georgieva N et al 2004, Georgieva N et al 2002).

Alpha tocopherol has been demonstrated to inhibit hepatocyte lipid peroxidation caused by agents like carbon tetrachloride (Martínez-Culva et al 1984) and halothane (Karakılçık AZ et al 2003). Halothane is a volatile anaesthetic that causes the formation of free radicals during its biotransformation Karakılçık AZ et al studied the hepatoprotective role of alpha-tocopherol in rats anesthetized with halothane. They administered alpha-tocopherol in a dose of 100 mg/kg, i.p. to rats and found that liver enzyme returned to near
control levels after vitamin E injections (Karakilcik AZ et al 2003).

However, there is inadequate data regarding the hepatoprotective effect of tocopherol in hepatotoxicity induced by anti tubercular drugs. So far there only one study which investigated the effectiveness of tocopherols and antihypoxic agents in INH, RMP and Pyrazinamide induced hepatotoxicity in rats, and has reported positive results (Skakun NP et al 1991).

Vitamin C is a water soluble versatile vitamin. It plays an important role in human health and disease. Vitamin C has become the most controversial vitamin in recent years. This is because of the claims and counter-claims on the use of Vitamin C in megadoses to cure everything from common cold to cancer.

Chemistry

Ascorbic acid is a hexose (6 carbon) derivative and closely resembles monosaccharides in structure. The acidic property of vitamin C is due to the enolic hydroxyl groups. It is a strong reducing agent. L-Ascorbic acid and undergoes oxidation to form dehydroascorbic acid and this reaction is reversible. Both ascorbic acid and dehydroascorbic acid are biologically active. However, D-ascorbic acid is inactive. The plasma and tissues predominantly contain ascorbic acid in the reduced form. The ratio of ascorbic acid to dehydroascorbic acid in many tissues is 15:1. On hydration, dehydroascorbic acid is irreversibly converted to 2, 3-diketogulonic acid which is inactive. Hydration reaction is almost spontaneous, in
alkaline or neutral solution. It is for this reason that oxidation of vitamin C is regarded as biological inactivation (formation of diketogulonic acid). Oxidation of ascorbic acid is rapid in the presence of copper. Hence vitamin C becomes inactive if the foods are prepared in copper vessels.

**Biosynthesis and Metabolism**

Many animals can synthesize ascorbic acid from glucose via uronic acid pathway. However, man, other primates, guinea pigs and bats cannot

synthesize ascorbic acid due to the deficiency of a single enzyme namely L-gulonolactone oxidase.

Vitamin C is rapidly absorbed from the intestine. It is not stored in the body to a significant extent. Ascorbic acid is excreted in urine as such, or as its metabolites-diketogulonic acid and oxalic acid.

**Pharmacodynamics**

In humans, an exogenous source of ascorbic acid is required for collagen formation and tissue repair. Vitamin C is a co-factor in many biological processes including the conversion of dopamine to noradrenaline, in the hydroxylation steps in the synthesis of adrenal steroid hormones, in tyrosine metabolism, in the conversion of folic acid to folinic acid, in carbohydrate metabolism, in the synthesis of lipids and proteins, in iron metabolism, in resistance to infection, and in cellular respiration. Vitamin C may act as a free oxygen radical scavenger *(Dolley 1991)*. The usefulness of the antioxidant
properties of vitamin C in reducing coronary heart disease was found not to be significant (Stampfer et al 1993; Rimm et al 1993).

To sum up, Tuberculosis is the leading cause of morbidity and mortality in developing countries, like India. Isoniazid and Rifampicin are the two most effective drugs for the treatment of Tuberculosis. However both these drugs have an associated potential of causing significant hepatotoxicity in a small percentage of patients. Although the exact mechanism of INH & RMP induced hepatotoxicity is not clear, recent studies have demonstrated the role of Cytochrome P450 in the generation of toxic metabolites from Isoniazid. Both electrophilic intermediates and free radical have been shown to be produced during Isoniazid metabolism. Since Rifampicin is a known inducer of Cyt P450, it further enhances the hepatotoxicity of INH.

Treatment of Tuberculosis in patients with antituberculosis-drug-induced hepatotoxicity poses a difficult clinical challenge. The culprit drug have to be curtailed and the treatment started with second line drugs which are not as efficacious as the first line drugs, are toxic and are also expensive during long term therapy.

Understandably, there is a great deal of interest in identifying therapies capable of reducing the risk of hepatotoxicity due to antitubercular drugs.
RECENT STUDIES

Ajay K et al (2009) showed that hepatotoxicity is one of the most important adverse drug reactions associated with antituberculosis drugs that may limit their use.

Bliven EE et al (2009) reviewed that Chronic Viral Hepatitis (CVH) was not established as a risk factor for INH hepatotoxicity during Latent Tuberculosis Infection (Luberculosis) treatment.

Fountain FF et al (2009) report suggests that hepatotoxicity is more likely in patients with baseline hepatic dysfunction and the need for increased vigilance in monitoring transaminases in these patients.

Prydenberg AR et al (2009) surveilled that adverse events will need to be improved when recommended dosages of the main first-line anti-tuberculosis therapy for children are increased.

Ho CC et al (2009) studied that abnormal baseline transaminase levels are the independent risk factors for anti-tuberculosis therapy-induced hepatitis.

Ozurk I.C et al (2009) presented the results which show that Vit C has a highly protective effect on hepatotoxicity and oxidative stress caused by CCl4.

Rajiv Nehra et al (2009) studied that the Isoniazid was found highly toxic for liver even in the first month of administration and the extent of toxicity is increased gradually and found highly significant. The Isoniazid was not found to create any significant effect on Renal Functions. But the Rifampicin was found toxic both for liver and
Kidney significantly, but not for initial 4-5 weeks.

**Sarda P et al (2009)** showed Acute Viral Hepatitis (AVH) in 14.7 per cent patients who developed hepatotoxicity while an antituberculosis treatment.

**Yue J et al (2009)** suggest that Rifampin co-administration does not increase Isoniazid-induced oxidative stress through hepatic CYP2E1 during short-term treatment in experimental rats. Rifampin co-administration significantly attenuated Isoniazid-induced CYP2E1 levels \( p<0.01 \) and inhibition of mu GST \( p<0.01 \). Rifampin did not increase the formation of DNA adducts induced by Isoniazid.

**Yue J et al (2009)** suggest that thiopronin exerts its hepatoprotective activity against Isoniazid-induced hepatotoxicity by inhibiting the production of free radicals in addition to its role as a scavenger. Thiopronin may reduce free radical generation via inhibition of hepatic CYP2E1 and increase the removal of free radicals directly or through the induction of cytosolic GSTs.

**Ziakas PD et al (2009)** observed that rates of hepatotoxicity were lower for patients who received 4-month Rifampin therapy compared with the corresponding rates for patients who received 9-month Isoniazid therapy and Rifampin was associated with significant reduction in the risk of hepatotoxicity.

**Adhvarya MR et al (2008)** conducted a research study and shows that herbal formulation prevent hepatotoxicity significantly and improved the disease outcome as well as patient compliance
without any toxicity or side effects.

Gond NY et al (2008) studied that there was significant reversal of biochemical, histological and functional changes induced by Rifampicin treatment in rats by petroleum ether extract treatment, indicating promising hepatoprotective activity.

Liu Q et al (2008) reviewed that there is no reliable evidence to support prescription of drugs or herbs to prevent liver damage in people on Tuberculosis treatment.

Makhlouf HA et al (2008) Anti-Tuberculosis-DIH is not uncommon, needs early recognition and treatment, and is more in patients with pre-existing liver disease and low BMI.

Marzuki OA et al (2008) pointed out that the prevalence of hepatitis was 9.7%. The presence of HIV infection and extrapulmonary Tuberculosis were significant risk factors for the development of hepatitis.

MiriM EM et al (2008) presented a case report of 76 year old man who was on daily dose of Isoniazid, Rifampicin and Pyrazinamide, laboratory data showed acute renal failure and discontinuation of Rifampicin results in normal renal function.

Pal R et al (2008) showed minimum dose with maximum hepatoprotection, indicated by return to normal of liver transaminase level. There was no further protective effect seen by increasing and decreasing the dose of carotenoids. The hepatoprotective nature of carotenoids in INH+RIF treated rats may be attributed to their
antioxidative property.

**Shan C et al (2008)** reported that the difference in induction of CYP 2E1 by Rifampicin between rat and human hepatocytes accounted for the difference in exacerbation of Isoniazid hepatocyte toxicity by Rifampicin, with more significant toxicity in gel entrapment than in monolayer culture.

**Srivastava R K et al (2008)** pointed out that insulin-treated diabetic animals showed greater silymarin-induced hepatoprotection against ATD-induced liver injury, which was characterized by near normal levels of marker enzymes, an increase in total proteins and normal hepatic structure.

**Sude E et al (2008)** carried out a research study and show that the active components of a silymarin had protective effects against hepatotoxic action of drugs used in the chemotherapy of Tuberculosis in animal models.

**Taki H et al (2008)** observed that the average serum uric acid concentration before Pyrazinamide treatment was 4.73 +/- 1.78 mg/dl, while the average uric acid level after Pyrazinamide treatment was 10.63 +/- 2.67 mg/dl, which was significantly higher than the pretreatment level (p<0.0001).

**Tandon VR et al (2008)** showed that Hepatoprotective (HP) activity of Vitex negundo (VN) leaf ethanolic extract was investigated against hepatotoxicity (HT) produced by administering a combination of three anti-tubercular drugs Isoniazid (INH)-7.5 mg/kg, Rifampin
(RMP)-10 mg/kg and Pyrazinamide (PZA)-35 mg/kg for 35 days by oral route in rats. There was a significant decrease in AST, ALT and ALP levels in comparison to control.

Tostmann A et al (2008) reported that incidence of anti Tuberculosis drug-induced hepatotoxicity, the most serious and potentially fatal adverse reaction, varies between 2% and 28%.

Yimer G et al (2008) concluded that anti-tuberculosis DIH is a major problem in HIV-associated tuberculosis with a decline in immune status and that there is a need for a regular biochemical and clinical follow up for those patients who are at risk.

Adhvarya MR et al (2007) found curcuma longa and tinospora cordifolia offer protection in guinea pig model of ATT induced hepatotoxicity.

Chang KC et al (2007) conducted a case control study and examine that age >49 years increase the risk of drug induced hepatitis.

Cho YJ et al (2007) conducted a research study on “Tuberculosis in patients with liver Cirrhosis” and shows Rifampicin and Isoniazid induces the hepatotoxicity.

Di Sario A et al (2007) showed that treatment with vitamin E or other antioxidant compounds could be proposed for nonalcoholic fatty liver disease (NAFLD), the most frequent hepatic lesion in western countries which can progress to nonalcoholic steatohepatitis and cirrhosis due to the production of large amounts of oxidative
stress products.

**Gupta S et al (2007)** showed that Hepatotoxicity and hyperuricaemia are seen after administration of Pyrazinamide. The drug inhibits elimination of urates resulting in hyperuricaemia. They reported bilateral leg cramps due to hyperuricaemia following Pyrazinamide therapy.

**Harada Y et al (2007)** reported a rare case of fatal liver failure due to anti tuberculous therapy.

**Khalid M et al (2007)** stated that most of the patients (61%) developed the hepatotoxicity within two weeks of starting anti-tuberculosis therapy with mild to moderate alteration in ALT and AST. AIT-induced hepatitis is significantly more frequent and more severe in patients with hepatotoxicity risk factors.

**Markov M et al (2007)** conclude a research study and shows anti-tubercular drug therapy induces the liver injury.

**Tasduq SA et al (2007)** observed that Pyrazinamide did not promote lipid peroxidation, and has no effect on antioxidant status in the liver, either when used in alone or with INH and /or RIF.

**Vandana T et al (2007)** produced a successful model of hepatotoxicity in albino rabbits by giving Isoniazid and Rifampicin in combination, her result revealed that pre treatment with high dose of tocopherol prevent hepatic damage induced by INH and RMP combination.
Anand A et al (2006) supported that age is not a risk factor for development of hepatotoxicity in patients taking anti-tuberculosis therapy.

Bhupinder SK et al (2006) reported that cimetidine in high dose can prevent hepatotoxicity induced by Isoniazid-Rifampicin combination.

Kheirollah G et al (2006) studied that anti tubercular drug induced adverse reactions and the most frequent system organ class affected by ADRs was liver and biliary system. The most serious adverse reaction was hepatitis leading to death in two patients.

Lengyel G et al (2006) showed that Oxidative stress is able to enhance the progression of chronic inflammatory liver disease independently from the aetiological factor or with it together. That is why the antioxidant drugs could be applied also in the treatment of chronic liver diseases beside the therapy based on the aetiological factors.

Mauro S et al (2006) pointed out that Rifapentine/Isoniazid was better tolerated than Rifampin/Pyrazinamide and was associated with good protection against Tuberculosis.

Oyinbo CA et al (2006) observed that treatment with vitamin C and E protects the hepatocytes and reduce the severity of damage due to ethanol toxicity.


Spigelman M et al (2006) and Johnson JL et al (2006) pointed out that new regimens are in development, with emphasis on fluoroquinolones such as moxifloxacin and levofloxacin.

Vuilleumier N et al (2006) and Huang YS et al (2003) showed in his human genetic studies that cytochrome P450 2E1 (CYP2E1) is involved in Anti-Tuberculosis drug-induced hepatotoxicity (ATDH).


Choi SH et al (2005) report a case of patient who was treated in Allopurinol and he was presented with worsening renal function and vanishing bile duct syndrome.

Fountain FF et al (2005) showed that increasing experimental evidence suggest that elevations of transaminase are reported in patients treated with Isoniazid monotherapy.

Halliwell B et al (2005) stated that Foods and beverages rich in phenolic compounds, especially flavonoids, have often been associated with decreased risk of developing several diseases. Tocopherols and
tocothenols may also exert direct beneficial effects in the gastrointestinal tract and that their return to the gastrointestinal tract by the liver through the bile may be physiologically advantageous.

Hathcock et al (2005) reported that the data consistently shows the safety of both the Vitamins C and E or the combination of the two.

Huang HY et al (2005) conducted a double-blinded placebo-controlled randomized trial in research units and showed that serum uric acid levels were significantly reduced in the Vitamin C group.

Kwara A et al (2005) shows that Rifampicin also interacts with antiretroviral drugs and effects the plasma levels of these drugs as well as risk of hepatotoxicity.

Marra F et al (2005) reported that new regimen levofloxacin will probably have lower toxicity rates.

Pan L et al (2005) observed that there is no association between Anti-HCV and HBsAg positivity and development of hepatotoxicity.

Rekha VVB et al (2005) reported that during re-treatment with an intermittent Rifampicin containing regimen in 3 patients who had been previously treated with a daily Rifampicin regimen, encountered acute renal failure.

Tasduq SA et al (2005) studies indicate the existence of a strong correlation between hepatic injury and oxidant stress in experimental animals treated with anti tuberculosis drugs.
Tost JR et al (2005) and Sharifzadesh M et al (2005) evaluate that when treatment is not interrupted in time, ATDH can be fatal.

Wilkinson GR et al (2005) stated that genetic polymorphism in drug metabolizing enzymes can affect enzyme activity. This may cause differences in treatment response or drug toxicity, due to an increased formation of reactive metabolites.

Younossian AB et al (2005) showed in his study that seven out of 12 patients (58%) treated for latent tuberculosis with Ethambutol and Pyrazinamide developed transaminase elevation of more than four times the upper limit of normal.

Campos-Franco JA (2004) Kimmoun E et al (2002) and Cheuk-ming T (2002) studies showed transient elevations of serum hepatocellular enzymes (e.g. alanine aminotransferase and aspartate aminotransferase) in approximately 10% of patients who received a standard combination chemotherapy including isoniazid and Rifampicin, of these 1-2 % patients withdrew from the treatment because of severe hepatotoxicity that ultimately led to fulminant hepatitis. Although the occurrence of drug induced hepatotoxicity is difficult to predict, it has been observed that certain patients are at higher risk during the course of anti-tuberculosis chemotherapy.

Fernandez-Villar A et al (2004) and Telemann MD et al (2002) stated that among the most widely accepted risk factors for ATDH are advanced age (above 60 years), female sex and low body mass index or malnutrition.
Hagymasi K et al (2004) Stated that combined antioxidant treatment is more favourable compared with monotherapy, because antioxidants have scavenger-compartment and tissue-specificity and they regenerate each other directly, too. Beside their antioxidant property they may also directly regulate many important processes, like cell cycle.

Jindani A et al (2004) observed that daily treatment may be more hepatotoxic than thrice weekly treatment involving combination therapy with Isoniazid, Rifampicin and Pyrazinamide.

Knowles SR et al (2004) showed that reactive metabolites, rather than parent drug are responsible for most idiosyncratic reaction.

Nedyalka G et al (2004) confirmed that there is an increased oxidative stress and decreased antioxidant defense factors in mice treated with INH. Results have demonstrated the tuberculostatic activity and SSA of the isonicotinoylhydrazones SH7 and SH8 results showed their hepatoprotective effect in mice.

Rajni S et al (2004) found that antitubercular drugs are associated with derangement of hepatic function resulting in elevation of liver enzyme. Risk factor of hepatotoxicity includes female gender, disease extent and poor nutritional status.

Reddy YN et al (2004) study showed that in tuberculosis patient free radical activity is quite high and antioxidant levels are low. Nutritional antioxidant supplementation may represent a novel
approach to fast recovery.

Shakya R et al (2004) pointed out that combination of Isoniazid with Rifampicin and Pyrazinamide increases the risk of ATT induced hepatotoxicity.

Sharma SK (2004) showed that the rate of hepatotoxicity is much higher in developing countries like India (8%-30%) compared to that in advanced counties.

Solangi GA et al (2004) observed in his study that Anti-tuberculous therapy with Pyrazinamide affects the uric acid levels early. This change is reversible after the withdrawal of the agent.

Villor AF et al (2004) reported the incidence of ATT induced hepatotoxicity is different in various countries though not fully understood but could be due to the characteristics and the risk factors of the population studied, the different diagnostic criteria used to define hepatotoxicity, different geographical areas, tests carried out during follow ups and the type of monitoring. Studies are denying the alcohol as a predisposing factor for ATT induced hepatotoxicity.

Yue J et al (2004) observed that the metabolite of INH, hydrazine, plays an important role in INH-induced hepatotoxicity in rats. The induction of CYP2E1 by hydrazine is involved in the hepatotoxicity of INH. Rifampicin does not exacerbate INH-induced hepatotoxicity in short term, which relates to down-regulation of CYP2E1.
Fenniche S et al (2003) and Burgess et al (2000) conducted a study and show that Rifampicin-induced renal failure is mediated by mechanisms of hypersensitivity type II or, less frequently, type III. It is hypothesized that anti-Rifampicin antibodies bind to the I antigen present on the cellular surface of adult erythrocytes, platelets, and renal tubular epithelium. This binding then induces cellular destruction.

Frieden TR et al (2003) stated that the most frequent adverse effects of antituberculosis treatment are hepatotoxicity, skin reactions, gastrointestinal and neurological disorders. Hepatotoxicity is the most serious one and is the focus of the present study.

Hussain Z et al (2003) stated that ATT inducible cytochrome P-450 2E1 (CYP2E1) is constitutively expressed in the liver. Studies show that polymorphism of the N-acetyltransferase2 (NAT2) genes and glutathione-s transferases (GST) are the two major susceptibility risk factors for ATT induced hepatotoxicity.

Lee McNeill et al (2003) pointed out that the risk of hepatitis in patients receiving Pyrazinamide/Rifampin for prevention of latent tuberculosis is increased threefold as compared to patients receiving Isoniazid.

Lee WM et al (2003) report that Isoniazid-induced hepatotoxicity is considered idiosyncratic. The exact mechanism is unknown.
Mitchael T et al (2003) showed in his studies that patients who were taking ATT when used alone or in combination with alcohol may increase the risk for hepatotoxicity in patients taking acetaminophen.

Tesebye M et al (2003) pointed out that in healthy Ethiopians, concentrations of antioxidants Vitamin C, vitamin E and vitamin A were significantly lower in tuberculosis patient and high malondialdehyde concentration were associated with clinical severity.

Yee D et al (2003) produced the data which suggest that incidence of Pyrazinamide induced hepatotoxicity was significantly higher than other first line anti-tubercular drugs. They also showed that female, HIV-infected, older and asian-born patients had greater risk.

Huang YS et al (2002) conducted a study in Hong Kong Chinese patients resulted in 13% risk of ATT induced hepatotoxicity and demonstrated that slow acetylators have a more than two-fold risk of developing ATDH compared with fast acetylators.

Lee AM et al (2002) carried out a public health program and shows that hepatotoxicity occurred in a high proportion of patients prescribed Pyrazinamide.

Muthukumar T et al (2002) reported a case which shows the deterioration in renal function typically appears acutely after reintroduction of Rifampicin. However, a few cases have described this adverse reaction as developing with continuous administration of the drug.
Attri S et al (2001), Attri S et al (2000) observed the hepatoprotective effect of N-acetylcysteine (a sulphhydryl-containing compound that can reduce oxidized glutathione into reduced glutathione) in rats treated with Isoniazid and Rifampicin further supports this involvement.


Choudhary A et al (2001) showed that oxidative stress could play a role in the pathogenesis of antitubercular drug (ATD) induce hepatotoxicity and lower levels of plasma glutathione and higher levels of MDA may be due to oxidative stress-resulting from ATD therapy.

Kiyota K et al (2001) reported a case of 19 year old girl who ingested Isoniazid and Ethambutol intentionally during day 4 and 5 liver dysfunction worsened and prothrombin time declined below 5%.

Tanaoglu K et al (2001) defined hepatotoxicity as normalization of liver function after withdrawal of all anti-tuberculosis drugs, and the presence of at least one of the following criteria: (1) appearance of jaundice (2) a rise of five times the upper limit of normal levels (50 IU/L) of serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT); (3) a rise in the level of serum total bilirubin > 1.5mg/dl.

Knekt et al (2000) showed that low Vitamin E status has been associated with an increased risk of rheumatoid arthritis.

Ohno M et al (2000) conducted the study in Japan to assess the role of age, sex, severity of the disease, nutritional status, alcoholism, use of paracetamol and effect of cholesterol as a risk factor for ATT induced hepatotoxicity.

Pereira RM et al (2000) report a case of a patient who developed toxic hepatitis accomplished by hepatic failure while he was being treated with Isoniazid, Pyrazinamide and Rifampicin. The recovery was fast when the use of Isoniazid was suspended.

Wong et al (2000) reported that drug-induced hepatotoxicity developed more frequently in HBsAg carriers than in noncarriers. In their study, drug-induced hepatotoxicity was defined as an increase in ALT levels to 1.5 times the upper normal limit, and for patients with increased pretreatment levels of ALT the ALT elevation had to be >1.5 times the baseline level.

Aithal PG et al (1999) showed that drug induced hepatotoxicity is best known toxic effect.

Charles M Nolan et al (1999) defined the risk factor morbidity and mortality of adverse events from Isoniazid particularly hepatotoxicity.

Morel et al (1999) studied the behaviour of Allopurinol on 60 yrs old man, after one month of the treatment his renal function,
previously normal, became severely impaired and dialysis was required. Hypersensitivity to Allopurinol was suspected.

**Ozturk (1999)** reported that people with rheumatoid arthritis have an impaired antioxidant system, making them more susceptible to free radical damage.


**Pereira S et al (1998)** report a case of lethal massive hepatic necrosis due to Allopurinol in a patient with the asymptomatic hyperuricemia.

**Pluim HJ et al (1998)** report a case of patient who was treated with Allopurinol and developed severe renal and liver dysfunction.

**Wittenborg et al (1998)** and **Kolars et al (1990)** conducted a double-blind trials (Using similar high levels of Vitamin E) reported that Vitamin E had approximately the same effectiveness in reducing symptoms of rheumatoid arthritis as anti-inflammatory drugs.

**Andrade RJ et al (1997)** report a case of 39 year old female who show progressive Jaundice and worsening of renal function 19 days after the initiation of treatment with Allopurinol.

**Changtham DC et al (1997)** present a case of young female with history of Isoniazid poisoning.

Edmonds et al (1997) and Michle et al (1997) studied in other double-blind trials, 600 IU of Vitamin E taken twice daily was significantly more effective than placebo in reducing RA. Although laboratory measures of inflammation remained unchanged.

Hwang et al (1997) reported in his prospective study that the occurrence of drug-induced hepatotoxicity during anti-tuberculosis treatment was found not to be significantly different between HBsAg and non-carriers. In this study drug-induced hepatotoxicity was defined as an elevation of ALT levels above the upper normal limit.

Durand F et al (1996) showed that Isoniazid and Pyrazinamide are major hepatotoxins. The remaining 2 agents (Rifampicin and Ethambutol) are rarely or not hepatotoxic. However, Rifampicin, which is a powerful enzyme inducer, may enhance the hepatotoxicity of Isoniazid.

Sarich TC et al (1996) pointed out the INH-induced hepatotoxicity manifests as hepatic necrosis.

Schaberg T et al (1996) pointed out that side effects (hepatotoxicity) of standard anti-tuberculosis therapy are frequent hospitalized patient aged >60 years or with a history of previous hepatitis and are probably due to Pyrazinamide rather than to Isoniazid or Rifampicin.

Corbella X et al (1995) showed that the mechanism of hepatotoxicity has been considered to be dose-related but in one case report rechallenge after an initial reaction to a combination regimen
lead to an increase in serum amino-transferases level to eighty times the upper limits of normal with an associated eosinophilia, suggestive of hypersensitivity reaction.

Schaberg T (1995) reported that only the frequency of clinically apparent hepatitis but not that of elevated liver enzymes or abnormal liver function tests, and have not commented on patients at risk of hepatotoxicity.

Buettner GR (1993) Studied Vitamin E is located in membranes and vitamin C is located in aqueous phases, vitamin C is able to recycle vitamin E; i.e., vitamin C repairs the tocopheroxyl (chromanoxyl) radical of vitamin E, thereby permitting vitamin E to function again as a free radical chain-breaking antioxidant.

Fairburn et al (1992) showed Vitamin E is an important antioxidant protecting many tissues, including joints, against oxidative damage. Low Vitamin E levels in the joint fluid of people with rheumatoid arthritis have been reported.

Kothari AK et al (1991) presented a case report of 29 year old male patient developed the secondary ichthyosis with normal daily dosage of Rifampicin.

Scherak O et al (1991) Studies carried out in a double-blind trial, approximately 1,800 IU per day of Vitamin E was found to reduce pain from rheumatoid arthritis.

Steele MA et al (1991) documented the adverse reaction to Rifampicin and Ethambutol.
Singhal KC et al (1990) observed that use of Rifampicin causes a symptomatic elevation of liver enzymes and jaundice. Ethambutol and Isoniazid was also found to cause ADRs.

Taneja DP et al (1990), Parthasarathy R et al (1986), Purohit SD et al (1983) reported that the high risk of hepatotoxicity is higher in Indian patients as compared to other countries of west.


Íñigo García-Roché M. O. et al (1987) pointed out that daily doses of ascorbic acid [(211 ± 40) and (18 ± 4) mg/kg] seemed to hinder hepatotoxicity according to the criteria under investigation.

Niki E (1987) Stated that interaction between vitamin C and vitamin E radicals can take place not only in homogeneous solutions but also in liposomal membrane systems where vitamins C and E reside separately outside and within the membranes respectively, and vitamin C can act as a synergist.


Rugmini PS et al (1984) documented that Rifampicin appears to interfere mainly with uptake by an effect on the membrane receptor proteins of the hepatocyte, leading to elevated levels of unconjugated proteins.
Bartelink AK et al (1983) reported a case of 58 years old man and stated that the risk of hepatotoxicity from Isoniazid is increased when it is given concomitantly with anticonvulsants, halothane or rifampin, all of which induce microsomal enzymes.

L.W Whitehouse et al (1982) observed histopathological effects of Isoniazid on the livers of male SPF Newzealand white rabbits following subacute administration of INH.

Mitchell MC et al (1981) observed the hepatoprotective effect of Cimetidine on Acetaminopen induced hepatotoxicity effect of Cimetidine on Acetaminopen induced hepatotoxicity in rats and the dose used was 120mg/kg, i.p.

Powell J et al (1981) has reported that patients taking both drugs have atleast 5 times the incidence of symptomatic hepatitis when compared with INH alone.

Thomas et al (1981) studied the induction of isoniazid metabolism by Rifampicin (100mg/kg, orally) pretreatment in rabbits.

Girling DJ (1978) stated that patients treated with Rifampicin monotherapy shows hepatotoxicity.

Chan HL et al (1977) shows a report on Allopurinol associated hypersensitivity reaction with acute and chronic renal failure.

Stein et al (1976) Pointed out that vitamin C enhances the Urinary excretion of Uric Acid.

Pozzi E et al (1974) observed that Rifampicin inhibits both uptake and excretion of bilirubin in a dose related manner giving rise
to elevated plasma levels of conjugated and unconjugated bilirubin without producing parenchymal injury.

Lal S et al (1972) studied that incidence of hepatotoxicity is higher with Isoniazid and Rifampicin combination than with Isoniazid alone.

Phillippe G et al (1972) report that Rifampicin when given alone rarely cause hepatitis.