The preservation of human life is of ultimate value, a pillar of ethics and the foundation of all morality. The last 25 years or so have seen the accumulation of a large body of evidence pointing to the relationship between the oxidative stress and human diseases. Biological oxidation and its pathological arm, the oxidative stress, should be regarded in the broader context of life's "rusting" process. This process, while natural and inevitable can be slowed down to a certain extent. As we fight the destructive action of oxygen on all sorts of man-made products so we must aggressively counteract excess free radical activity which can harm the body in so many ways. Antibiotics have been one of the most successful ways of saving human life ever discovered but these so called life savior poses an increasing threat to human health across the world. It accounts for approximately one-half of the cases of acute and chronic liver disease. Drugs account for 20-40% of all instances of fulminant hepatic failure (Kosanam and Boyina, 2015). Drug-induced hepatic injury is the most common reason cited for withdrawal of an approved drug. Drug resistance is a global threat and the irrational use of it has become deadly for human life as well. These drugs have transformed life and longevity and saved countless millions since penicillin was discovered by Sir Alexander Fleming in 1928 now saturate every corner of environment (The New Indian Express, 2013). The overuse and abuse of...
antibiotics have led to a state of ‘Pre-Alexander (Fleming) Era’. As of now, there is no antibiotic to kill drug resistant bacteria. In the current scenario, anti-microbials are the most widely used category of drugs in the world. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Antibiotic related liver injuries cover most of the clinical and pathological expressions of hepatic dysfunction, including cytotoxic hepatitis (isoniazid), intrahepatic cholestasis (macrolides, penicillins), mixed hepatitis (sulphonamides), chronic active hepatitis (nitrofurantoin) etc.

A growing international health concern is Tuberculosis caused by *Mycobacterium tuberculosis* which is the biggest killer among the infectious diseases in the world today. The first line anti-tuberculosis drug: Isoniazid, Rifampicin, Pyrazinamide and Ethambutaol continues to be the effective drugs in the treatment of tuberculosis, however, the use of these drugs is associated with toxic reactions in tissues, particularly in the liver, leading to hepatitis (Shukla et al., 2014).

**Global Scenario of Drug Induced Liver Injury**

- In US, approx 2000 acute liver failure cases occur annually 50% of them due to drugs (Pandit et al., 2012)
- WHO reported >9million new cases of TB globally (Jagielski et al., 2014)
- In developing countries like India rate of hepatotoxicity is much higher (8-30%) (Panda et al., 2015)
- India has the highest TB burden in the world, only next to China (Yang et al., 2014)
- Risk of DILI in active TB treatment range between 5 to 33% (Jong et al., 2013)
Antitubercular drug mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane (Lian et al., 2013). Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components (GSH) have been reported in INH and RIF-induced hepatotoxicity (Kumar et al., 2013; Verma et al., 2013). The combination of anti-tuberculosis drug namely Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PZA) are getting popularized in the treatment of tuberculosis (Somasundaram et al., 2014). In India, pulmonary tuberculosis is one of the major causes of the adult death. Drugs in the therapy, in addition to their role of killing bacteria effectively could also cause different kinds of adverse drug reactions (ADRs) such as hepatotoxic reaction, gastro-intestinal discomfort, drug allergy etc.

The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Therefore, the maintenance of a healthy liver is vital to overall health and well being. The liver is particularly exposed to drugs and their metabolites. It is prone to xenobiotic-induced injury because of its central role in xenobiotics metabolism, its portal location within the circulation, and its anatomic and physiologic structure (Hermenean et al., 2015; Manal et al., 2010). Large numbers of xenobiotics are reported to be potentially hepatotoxic. Free radicals generated from their metabolism can induce lesions of the liver and react with the basic cellular constituents such as proteins, lipids, RNA and DNA.

Drug-induced liver damage includes:

- Disruption of intracellular calcium homeostasis
- Cholestatic damage
- Interruption of transport pumps and loss of villous processes
- Reactions involving cytochrome P-450 system
- Activation of apoptotic pathways and programmed cell death
- Inhibition of mitochondrial function
- Induction of hepatic enzymes
- Drug-induced acute hepatitis

(Heidari et al., 2015; Kim et al., 2015; Ramappa et al., 2013)
Idiosyncratic reactions are the consequence of a “multihit process” due to the succession of unlikely events and are characterized by a variable latency period from the initial time of ingestion of the drug. Idiosyncratic reactions are frequently fatal if the drug is continued once the reaction has begun.

**Anti-tuberculosis Drug: Resistance and Hepatotoxicity**

It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV) (WHO, 2013). Tuberculosis is now about to come of age as a global emergency, this disease is aptly regarded as “humanity's greatest killer” and a serious and substantial issue internationally (Herbert et al., 2014).

Anti-tuberculosis drug induced liver injury (ATDILI) ranks the first in all kinds of drug-induced liver injuries. It is one of the most challenging clinical problems and main cause of treatment interruption during TB treatment course that causes hospitalization and life threatening event. First line anti-TB drug (INH, RIF and PZA) cause hepatotoxicity and the risk is enhanced when these drugs are used in combination (Shin et al., 2015). Different studies reported that of TB patients experience drug related hepatotoxicity following TB treatment ranging in severity from asymptomatic elevation of hepatic transaminases to hepatic failure necessitating liver transplantation (Dhingra et al., 2014; Shang et al., 2011).

The emergence and spread of Multi Drug-Resistant Tuberculosis (MDR-TB) and Extensively Drug-Resistant Tuberculosis (XDR-TB) are major public health problems that threaten progress made in TB care and control worldwide. MDR-TB is caused by bacteria that are resistant to at least rifampicin and isoniazid - the two main first-line anti-TB drug. The annual global MDR-TB burden is enormous and it is a challenge to TB control due to complex diagnostic and treatment requirements and it is important cause of mortality and morbidity (Gill et al., 2013; Balaji et al., 2010). XDR-TB is caused by bacteria that are resistant to rifampicin and isoniazid as well as resistant to any one of the fluoroquinolones (e.g. ofloxacin and moxifloxacin) and to at least one of the injectable second-line drug (capreomycin, viomycin, kanamycin or amikacin).
“Hepatotoxicity has been the most common single adverse effect causing major drug problems, including withdrawals and refusals to approve”

Bob Temple, M.D.
FDA

INH, RIF and PZA are essential components of the directly observed treatment short-course (DOTS) strategy for control of tuberculosis endorsed by the World Health Organization (WHO, 2014; Moonan et al., 2011) and all the three drugs have been observed to have hepatotoxic potential. Drugs in the therapy, in addition to their role of killing *Mycobacterium* effectively could also cause different kinds of adverse reactions (ADRs) such as hepatotoxic reaction, gastro-intestinal discomfort, drug allergy and severe outcomes like liver failure as well (Xia et al., 2010). These ADRs are regarded as one of the major cause of incompliance of anti-TB treatment and may lead to final termination of TB treatment. Anti-tubercular drug mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane.

**Epidemiology and Demographics of ATDILI**

- The incidence of drug induced liver injury is about 1/1000 to 1/10000 among patients who receive therapeutic drug doses (Kosanam and Boyina, 2015).
- In 2013, 9 million people fell ill with TB and 1.5 million died (WHO, 2015).
- 450 000 Multidrug-resistant tuberculosis in the world in 2012 (WHO, 2014).
- Antibiotic resistance, in India, where the burden of infectious disease is high and healthcare spending is low (The New Indian express, 2013).
- Brazil is one of 22 countries prioritized by the WHO that together account for 80% of the world’s TB cases (Lima and Melo, 2012).
- Childhood TB accounts for 6% to 10% of all TB cases worldwide. More than 74,000 children die from the disease each year (CDC, 2012).
- The 17th Global Report on Tuberculosis showed that there were an estimated 8.7 million new cases of TB worldwide in 2011 (WHO, 2012).
• Conventional medicine (herbs) is now pursuing the use of natural products to provide the support that the liver needs on a daily basis (Mannem, 2014; Phaneendra et al., 2011).

**Problems with current Anti TB Regime**

Despite the introduction 40 years ago of the inexpensive and effective four-drug (isoniazid, rifampicin, pyrazinamid and ethambutol) treatment regimen, tuberculosis remains to cause considerable morbidity and mortality worldwide. Anti-tuberculosis drug (ATD) are a two-edged sword. While they destroy pathogenic *Mycobacterium tuberculosis* they also induce liver injury which hamper as today’s TB drug regimen takes too long to be effective and requires too many medications.

Isoniazid is a central component of drug regimen used worldwide for the treatment of infections, it enters *M. tuberculosis* as a prodrug by passive diffusion and is activated by catalase-peroxidase, encoded by katG, to generate free radicals, which then attack multiple targets in the cells (Ajiboye et al., 2015). Rifampicin, a powerful inducer of mixed-function oxidase, increases the hepatotoxicity of INH by enhancing the production of toxic metabolites from acetylhydrazine (Kalra et al., 2007). Pyrazinamide is an essential front-line drug metabolized via pyrazinamidase (PZase) to its active form pyrazinoic acid (Via et al., 2015). PZA and its analog, 5-chloro-PZA, may inhibit the fatty acid synthetase I (FASI) enzyme of *M. tuberculosis* (Drew, 2014) disrupts membrane energetics and inhibits membrane transport function. Ethambutol is adjunctive anti-tuberculosis medication which is used only in combination with other agents. It has been associated with minor, transient and asymptomatic elevations in serum aminotransferases. Hepatotoxicity (DIH) is the most common side effect leading to interruption of therapy (Cicek et al., 2015; Singla et al., 2010) all across the world. This may be:

• Because of the long duration and associated side effects of standard TB drug regime, there is fosters emergence of single and multi-drug resistant TB (MDR-TB) strains.

• The treatment course for drug-resistant TB is even longer, sometimes more than two years. This therapy usually includes the use of costly second-line
drugs that are often less effective and more toxic. In addition, patients with drug-resistant TB may remain infectious for long periods, thereby increasing the chance for transmission of resistant strains.

- The failure of treatment with first-line drug regimens requires the use of second line drug; however, some circulating strains are resistant to these second-line drug as well, and these extensively drug-resistant strains are very disturbing (Ge et al., 2010).

Drugs to protect the liver are frequently prescribed in some countries as part of treatment for tuberculosis. Currently, there is no effective treatment for antitubercular drugs mediated hepatotoxicity. Approximately 80% of world population depends exclusively on plants for their health and healing. In the recent years people are complimenting their treatment with natural supplements (Prabu and Lakshmipathy, 2012). Many ayurvedic herbs have a long history of traditional use in revitalizing the liver and treating liver dysfunction and disease. Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver toxicity and may prove to be valuable sources of new anti mycobacterials as well (Hussain et al., 2014; Santhosh and Suriyanarayanan, 2014). By limiting the action of free radicals in biological systems and making appropriate life style changes we can actually prevent many pathological conditions from occurring. This new strategy that begins to get more and more attention from the medical establishment is called prevention and could result in savings of billions of dollars for an already over stretched medicare system.

Benjamin Franklin once said:

"An ounce of prevention is worth a pound of cure"

As never before are these words more true than today. That is why, the preventive medicine together with a host of other measures will play center stage in the fight against disease. This could bring marked improvements in the quality of life for millions of people as well as benefits to society as a whole.
Traditional medicine is still stronghold of more than 50% of the world population, especially in developing countries. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Jerang et al., 2015).

**Green medicine as new paradigm**

India, having a pluralistic healthcare system, offers an unfettered choice for new clinical effects of traditionally used medicinal plants. Indian medicinal plants are rich source for health care moieties to prevent different states of disease. In spite of the great progress observed in modern medicinal systems in recent decades, herbal drugs still make an important involvement to health care (Kiran et al., 2011). A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as **botanical garden** of the world (Saisri, 2015). In the last few decades there has been an exponential growth in the field of herbal medicine which is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. Recent years have seen considerable advances in our understanding of natural-product biosynthesis (Banu et al., 2013).

*Phyllanthus amarus* Linn. belongs to the family Euphorbiaceae, grows as small annual herb in India and other tropical regions of the world. It is commonly known as *bhumiamla* (Husain et al., 2015) Jăr amlă or Jangliamla, stonebreaker, carry me seed, windbreaker, gulf leaf flower or gala of wind (Pandurangan et al., 2015). It is a broad spectrum medicinal plant that traditionally used to treat flu, dropsy, diabetes, and jaundice arthritis, skin ulcer, dieresis, hepatic and urolitic diseases (Verma et al., 2014; Kiran et al., 2011). Phytochemical analysis of *Phyllanthus*
*Phyllanthus amarus* extract showed that primarily it contains lignans (phyllanthin and hypophyllanthin), flavanoids (quercertin, astralgin, rutin), tannins and alkaloids (Kwaji *et al.*, 2015).

**Phyllanthin** is one of the pharmacologically active constituents which possesses anti-inflammatory and analgesic action (Priya and Poonguzhal, 2015; Adedapo and Ofuegbe, 2013; Annamalai and Lakshmi, 2009). Thus present study will be focused on therapeutic effectiveness of *Phyllanthus amarus* and Phyllanthin on hepatic injury caused by anti-tuberculosis drug.

**Aim:**

*Scientific validation of Phyllanthus amarus and its active principle on anti-tuberculosis drug induced hepatic injury at biochemical and molecular level, to develop natural herb as a candidate therapy for TB patients.*

**Objectives:**

The present study was a meticulous endeavor with the following objectives:

- Evaluation of antioxidant potential of plant extract
- Restoration of membrane integrity of hepatocytes
- Detoxification strategy using drug metabolizing enzyme cytochrome P - 450
- Measurement of modulating potential of effective therapy on cytokines production during experimental regimen
- Revitalization effect on DNA damage
- To understand mechanism of apoptosis
- Recovery pattern in cell viability of Hep-G2 line
- To assess the degree of recovery pattern by observing histopathological and ultrastructural alterations

A thorough, scientifically based study was conducted to arrive at vital inferences, intended to reveal therapeutics advancements for the benefit of mankind.
**Expected Outcome:**

Chronic hepatic diseases stand as one of the foremost health troubles worldwide. The utilization of multidrug regimens for the treatment of TB such as the combination of INH, RIF, and PZA has been associated with an increased incidence of drug induced hepatotoxicity. Therefore, herbal medicine *Phyllanthus amarus* (PA) or its active principle Phyllanthin (PY) can be co-administered with anti-TB drug during the course of treatment. The present investigation on hepatoprotective nature of *Phyllanthus amarus* and Phyllanthin will strengthen our understanding on its mechanism of action and thus, may lead to the development of cost effective hepatoprotective drug to cure liver disorders which is the major side effect induced by of anti TB drug. Results of this study will help to unveil that PA/PY could afford a significant protection in the alleviation of ATD induced hepatic damage and could overcome the quest for development new biodynamic substances. Thus, this may result in completing the scheduled treatment of tuberculosis.