Introduction
1. Introduction

Tuberculosis (TB), one of the oldest recorded human afflictions is still one of the biggest killers among the infectious diseases, despite the worldwide use of a live attenuated vaccine and dozen of antibiotics, tuberculosis is a serious challenge to global health that kills one human every 15 seconds worldwide (Dye et al. 2005) and one Indian every minute (RNTCP 2009). This huge statistics is quite higher by any other disease to kill human being. Evident from the alarmingly high mortality (1.6 million lives annually) and morbidity (around 8.8 million new cases every year) (WHO 2009). TB is far from control, though effective chemotherapy is available for nearly half a century. This rude less march of TB is going on worldwide, fueled by the emergence of drug-resistant strains of TB and by increasing number of vulnerable people infected with human immunodeficiency virus (HIV). The strong synergy of HIV-TB has caused havoc in Africa and continues imposing serious challenges to public health at global level.

Owing to this alarming situation, World Health Organization (WHO) declared tuberculosis a “global health emergency” in 1993 the only disease in history ever so dignified. An untreated patient with active TB infects 10-15 persons on an average every year and one person somewhere in our world contracts the disease every second. The motto of World Tuberculosis Day 2007 (TB anywhere is TB everywhere), 2008 (I am stopping TB) and 2009 (Simply stopping TB) are the somber reminders of the need of combined and concerted global efforts required for the control of this global menace.

One third of the global population is latently infected with this organism and has a 5-10% risk of developing TB over their lifetime (Harries and Dye 2006). However, the annual risk of developing tuberculosis is 10 times higher in individuals with HIV co-infection. Two lakh people with HIV/AIDS die from TB every year. India shares 20% of the global burden of tuberculosis. About 1.8 million people develop the disease every year and nearly 370,000 die because of it annually i.e. more than 1000/day (RNTCP 2008; WHO 2009).

The discovery of first anti-tuberculosis drug, Streptomycin (STM) in 1944 by SA Waksman was the landmark step towards the chemotherapy of TB. With the subsequent discovery of Rifampicin (RIF), Isoniazid (INH), Ethambutol (ETM), Pyrazinamide (PZA) the basic Anti-TB arsenal was constituted and the combination therapy became the rule of TB chemotherapy. The current short course chemotherapy of tuberculosis for previously untreated cases includes two months of intensive phase with Rifampicin,
Isoniazid, Pyrazinamide and Ethambutol or Streptomycin followed by 'continuation phase' for next 4 months with RIF and INH only.

Rifampicin (RIF), a broad spectrum antibiotic discovered in 1963 and introduced to TB chemotherapy in 1971 was the most potent anti TB drug which make the sputum culture negative for M. tuberculosis in present available drug regimen during short course chemotherapy. The role of RIF as a sterilizing agent has been considered undoubtedly very significant, as it acts against semi-dormant tubercle bacilli, thereby reducing the chances of relapse and thus reducing the treatment period from previous 9 months to current 6 months regimen known as Directly Observed Therapy Short course (DOTS) (Mitchison 1985). This lengthy “short course” is imposed by the exceptionally slow growth of M. tuberculosis and its ability to persist within the host for long periods without causing any overt disease symptoms. It is also this long duration of the treatment which makes the patient compliance difficult, thereby contributing to the selection of drug resistant mutants among the predominantly susceptible bacterial population as a result of the inadequate regimens/interrupted treatment etc.

Rifampicin discovered 50 years back and is the backbone of modern anti-TB chemotherapy by virtue of being active against M. tuberculosis in exponential growth phase as well as possessing activity against non replicating persistent bacilli. Rifampicin inhibits β-subunit of DNA dependent RNA polymerase of rpoB gene. Any mutation in rpoB gene leads to rifampicin resistance, although mutations other than rpoB gene were also reported for rifampicin resistance (Ohno et al. 1996). Mutations in the rpoB gene of Mycobacterium tuberculosis are associated with degree of rifampicin resistance. More recently, the sequencing of the rpoB gene in M. tuberculosis and the development of direct sequencing of PCR products have allowed determination of the actual mutations (Miller et al. 1994; Telenti et al. 1993) and there has been extensive analysis of mutations in the rpoB gene of rifampicin-resistant patient isolates of M. tuberculosis (Morlock et al. 2000). Telenti et al. demonstrated that at least 95% of rifampicin resistant isolates have mutations in rpoB and that mutations are clustered in an 81-bp region (Telenti et al. 1993). M. tuberculosis resistance to Rifampicin is considered as surrogate marker to MDR M. tuberculosis as 90% of rifampicin resistance are also resistance to Isoniazid (Rattan et al. 1999).

Rifampin-based regimens are less effective when the standard 600-mg daily dose is administered twice- or thrice-weekly as opposed to daily (Chang et al. 2006) and
attempts to increase the rifampin dose to 1,200 mg or more in once- or twice-weekly regimens frequently result in an influenza-like syndrome (Grosset et al. 1983; Poole et al. 1971). Major limitations to rifampicin use are believed to include its short half-life ($t^{1/2}$) which allows cycles of $M. tuberculosis$ growth and resistance emergence. The main target organs are the liver and the gastrointestinal system. It is widely believed that no one anti-TB drug can prevent resistance to itself and however, the occurrence of drug resistance is often multifaceted and may include not only chromosomal mutations but also induction or the presence of efflux pumps. (Gumbo et al. 2007).

Antibiotics, the keystone of control strategies are becoming ineffective due to the rapid development of multi-drug resistant (MDR). MDR-TB is a laboratory diagnosis of resistance *Mycobacterium tuberculosis* to atleast isoniazid and rifampicin as well as Extensive drug resistant (XDR) currently defined as MDR-TB with further resistance to at least 3 of the 6 major classes of second line drugs. (Anon 2006; Nelson et al. 2005; Nunes et al. 2005). Although, Second-line anti-tuberculosis agents are less efficacious and more toxic than first-line drugs (Chambers et al. 2005).

In recent years, considerable progress has been made in understanding the drug resistance mechanisms in mycobacteria and it has led to the identification of some structural genes whose mutations lead to drug resistance. Recent evidence suggests that drug resistance to one or several compounds to *M. tuberculosis* is associated with constitutive or inducible expression of efflux systems (Danilchanka et al. 2008). The genome of *M. tuberculosis* strain H37Rv has 20 open reading frames (ORFs) encoding putative efflux proteins (Cole et al. 1998).

Although multidrug transmembrane protein (efflux pump) of *M. tuberculosis* are also associated with low level of drug resistance by inhibiting the influx of drugs inside the cell leading to drug ineffective in killing mycobacteria. One such ORF, Rv1410c in the genome of *Mycobacterium tuberculosis* (H37Rv) annotated as probable drug efflux protein and its ORF has been found to be identical to P55 gene in *M. bovis* which has been characterized (Bigi et al. 1997, 2000; Cole et al. 1998). The *M. fortuitum* tap efflux pump and its *M. tuberculosis* Rv1258c homologue confer resistance to tetracycline and aminoglycosides including streptomycin, a major drug in TB treatment (Ainsa et al. 1998). Deletion of the Rv1258c gene from the *M. bovis* BCG chromosome increased susceptibility to these two drugs confirming the involvement of this efflux pump in the intrinsic resistance of *M. bovis* and *M. tuberculosis* to tetracycline and streptomycin (De Rossi et al. 2005). This gene when cloned in *M. smegmatis* conferred low level
resistance to tetracycline (Ainsa et al. 1998). A correlation was recently identified between drug resistance and Rv1258c gene transcription levels in clinical M. tuberculosis isolate resistant to rifampicin and ofloxacin (Siddiqi et al. 2004). Also overexpression of Rv1258c has been reported in clinical isolates resistant to rifampicin (Jiang et al. 2008).

One of the ways to improve the efficacy of the present drugs is to use them in combination with some molecules which enhance the killing of mycobacteria more effectively and also prevents the emergence of drug resistance (Amaral et al. 2007). This can be achieved by addressing any of three approaches 1) Increasing the bioavailability of drugs in the host; 2) Inhibiting the drug efflux mechanism of the pathogen; 3) Up-regulating the host immune response.

Approximately 90 to 95% of initial infections are controlled by the cell-mediated immune response. However, TB immunity is static (Nathan and Shiloh 2000) and a residual population of viable bacteria may be maintained in a poorly understood state of clinical latency for extended periods (WHO 2005). Little is known of the early interactions of microbes and immune cells that result in either restricted infection or dissemination and disease, nor of the reasons why some individuals reactivate latent infection. However, cell-mediated immunity is critical for restricting M. tuberculosis infection; this is highlighted by the increased risk of tuberculosis associated with decreased cellular immunity such as by immunosuppressive drugs, certain cancers, and the acquired immunodeficiency syndrome. Anti-tuberculous cellular immunity involves the critical interplay of T lymphocytes, macrophages and cytokines (Chan and Kaufmann 1994; Ho et al. 1997). Mycobacterium-specific CD4+ and CD8+ T lymphocytes have been identified that have cytolytic activity against mycobacteria-harboring macrophages (Boom et al. 1991; Chan and Kaufmann 1994; Del et al. 1991a, 1991b; Orme et al. 1993). In mice infected with Mtb complex, depletion of CD4 T lymphocytes results in disseminated disease while competent mice have restricted infection (Boom et al. 1991; Chan and Kaufmann 1994). Similarly, when CD4+ T cell counts decrease in HIV-1-infected persons, the risk of tuberculosis is increased whether from primary infection or from reactivation of latent Mtb infection (Selwyn et al. 1992). In contrast to CD4+ T lymphocytes, the role of Mtb- or Mycobacterium avium-specific CD8 T cells in experimental murine infection and in humans remains undefined (Chan and Kaufmann 1994; Ho et al. 1997).
In the recent years researcher working on *M. tuberculosis* tried a number of drug combination with different concentrations to stop TB which involved large number of first and second line of drugs of TB regime (Alvireaz *et al.* 2002).

*Piper longum,* an important medicinal plant is widely used in traditional medicine by many people in Asia and Pacific islands especially in Indian Ayurvedic medicine. *Piper longum* is a major component of medicines reported as good remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain and arthritic conditions (Singh *et al.* 1986). Piperine, a major constituent of black pepper can inhibits human p-glycoprotein and CYP3A4. Piperine is known as bioavailability enhancer (Atal *et al.* 1981, 1985) and has potential for immunomodulatory activity (Sunila and Kuttan 2003). Recently piperine has also been reported as inhibitor of NorA efflux pump of *Staphylococcus aureus* (Khan *et al.* 2006; Kumar *et al.* 2008).

The work embodied in this thesis elucidates the role of piperine as mycobacterial efflux pump inhibitor. It was also evaluated as an immunomodulator of selective Th1 response in mice. The study further evaluated the potentiating effect of piperine in augmenting the bioefficacy of rifampicin in a mice model of *M. tuberculosis* infection.