Summary and Conclusion.

Despite stringent control strategies and many advances in our knowledge of the epidemiology of tuberculosis (TB) and the biology of the causative agent *Mycobacterium tuberculosis*, TB still remains one of the most common and deadly infectious diseases worldwide. The emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant (XDR-TB) are a major concern in the control of the global TB epidemic in today’s time.

In present study efficacy of rifampicin in combination with pipeline against *M. tuberculosis* isolates was evaluated using checkerboard method. The MIC of rifampicin was reduced by four to eight folds in the presence of pipeline. A combination of rifampicin and pipeline exhibited better bactericidal activity as compared to rifampicin alone during time kill studies. The most significant finding of this study is the ability of pipeline to prevent the emergence of rifampicin resistant mutants at a clinically achievable concentration of rifampicin. Another interesting aspect of this study was the extended PAE of rifampicin when tested in combination with pipeline. The PAE of a drug against *M. tuberculosis* might be a putative pharmacodynamic parameter that would be helpful in the designing of an optimal dosing schedule for an antimicrobial agent. Since efflux is the only known mechanism of resistance for ethidium bromide, reversal in MIC of ethidium bromide by any substance is an indication that the substance probably works as an inhibitor of bacterial efflux pumps. It was observed that the increase in the MIC of ethidium bromide in *M. tuberculosis* rif was reversed in presence of pipeline thus leading to the inhibition of efflux mechanisms. The qRT-PCR analysis of Rv1258c in *M. tuberculosis* rif grown in presence of rifampicin (¼ MIC) showed 3.6-fold increase in the transcription level of this gene as compared to bacilli grown without rifampicin. The Rv1258c is a putative efflux pump of *M. tuberculosis* and it is reported to be overexpressed in the rifampicin resistant clinical isolates. The crystal structure of Rv1258c is not available, 3-D structure of the protein was predicted based on homology modelling using in-silico approach. The docking studies revealed that one of the binding pockets showed greater affinity for pipeline when compared to known inhibitors like reserpine.

In the present study, we also investigated the immunomodulatory potential of pipeline mediated through the up-regulation of Th-1 immune response and its protective efficacy in a murine model of *M. tuberculosis* infection. The mice splenocytes exposed to
piperine exhibited proliferation of T and B cell, increased Th1 cytokines and enhanced macrophage activation. The maximum immunomodulatory effect was observed at 1 μg/ml. There was also no increase in the production of IL-4, a Th2 cytokine. Furthermore, piperine (10 mg/kg) in mice infected with *M. tuberculosis* activated the differentiation of T cells into Th1 sub-population (CD4⁺/CD8⁻ subsets). There was subsequent increase in secretion of Th1 cytokines (IFN-γ and IL-2) by these cells. During prophylactic study, after 2 months of infection there was 2 log increase in CFU/lung in untreated mice (late control) with respect to early control whereas, piperine at 10 mg/kg exhibited some protective efficacy and did not allow the infection to proliferate. The qRT-PCR studies revealed corresponding increases in the mRNA transcripts of IFN-γ and IL-2 in the infected lung tissues. Lung sections of the mice treated with piperine (10 mg/kg) showed well formed epitheloid cell granulomas during histopathology studies. In order to investigate the effect of piperine on the therapeutic outcome of rifampicin treatment, the *M. tuberculosis* infected mice were treated with a combination of piperine and rifampicin. There was marked improvement in the therapeutic outcome of rifampicin (10 mg/kg) when combined with piperine at (10 mg/kg). The combination of piperine and rifampicin (10 mg/kg) exhibited better efficacy of and resulted in additional 1.4 to 0.8 log reduction in lung CFU as compared to rifampicin alone, whereas the combination of higher dose of rifampicin (20 mg/kg) and piperine reduced the bacterial load in lungs below the detection limit within 4 weeks.

In conclusion, to our knowledge, this study provides the first evidence of piperine as an inhibitor of *M. tuberculosis* efflux pump Rv1258c. The inhibition of this pump resulted in the potentiation of rifampicin’s activity. In our study, we have for the first time reported the detailed immunomodulatory activity of piperine with reference to its protective immunity mediated through Th1 cytokines and its enhancement of the activity of rifampicin. Piperine in combination with rifampicin might improve its therapeutic efficacy in immuno-compromised TB patients.