Pyrazinamide Drug Resistant *Mycobacterium tuberculosis* In HIV Patients & Antituberculosis Activity of *Withania Somnifera* Dunal on Resistant Strains

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ABSTRACT

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* affecting nearly one-third of the global population. Transmission of multidrug – resistant strains of *Mycobacterium tuberculosis* (MDR-TB) presents a serious problem for TB control, particularly in the context of co-infection with the human immunodeficiency virus (HIV). MDR-TB has been well studied in outbreaks in settings of low endemicity in developed countries. However, the characteristics of MDR-TB in the community with high endemicity such as India have not been well investigated. This study was designed to isolate and characterise *pncA* gene from a clinical isolate of pyrazinamide drug resistant TB patients co-infected with HIV and to analyse the antituberculosis activity of *Withania somnifera* Dunal on the pyrazinamide drug resistant strains of M.tb. The blood sample obtained was first subjected to CD4 analysis using Partec Flow Cytometry for RT-PCR analysis to confirm HIV positive. The serum albumin was separated by 10% SDS – PAGE and then the suspected protein spot was sequenced using Nano LC/MS. The DNA was isolated from the pyrazinamide resistant *M. tuberculosis* culture, which was used for the amplification of the *pnc A* gene using primers by PCR technique. The PCR product was subjected to electrophoresis on 2% agarose gel and desired gene product was eluted by the gel cleanup kit. The *pncA* gene PCR purified products were analysed in the bioanalyser electrophenerogram to detect the molecular weight and was directly sequenced at Bioserve, Bangalore. The BLASTn and BLASTx search tool was used to compare the suspected DNA sequence for detecting the mutation to confirm the drug resistance. Then the pure culture of *M. tuberculosis* was then subjected to phylogenetic analysis using 16s rRNA primer by PCR technique. The 16s rRNA PCR purified products was sequenced in the automated DNA sequencer. The MEGA search tool was used to compare and differentiate the 16s rRNA sequence from H_{37}Rv wild strain and other *Mycobacterium* sp. Treatment of tuberculosis is more difficult in immunodeficient patients such as those infected with HIV due to the resistance problem. The hexane and methanolic extracts of herbal plant *Withania somnifera* Dunal were assayed in vitro for antitubercular activity against M. tuberculosis H37Rv and clinical isolates of pyrazinamide resistant M. tuberculosis. The antimycobacterial activity and percentage reduction in relative light units (RLU) were

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calculated using luciferase reporter phage (LRP) assay showed the highest inhibition of the resistant spp with *Withania somnifera* methanolic extracts.

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