TITLE: PIPERINE AS ADJUNCTIVE THERAPHY FOR TUBERCULOSIS

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ABSTRACT:

Tuberculosis (TB) still is a great public health problem in the world. The increase of resistant TB makes the search for new or alternatives treatment to be essential. Recently, bacterial efflux pumps (BEs), which can mediate resistance to drugs, have been described in mycobacteria. The existence of compounds capable of inhibiting *in vitro* BEs activity have also been demonstrated in causing considerably antimycobacterials minimum inhibitory concentrations (MICs) decrease when combined with BEs inhibitors. In this context Piperine (PIP), a compound extracted from the *Piper* genus, has been studied as a BE inhibitor in other microorganisms such as *Staphylococcus aureus* and *Mycobacterium smegmatis*. Thus, the objective of this study was to evaluate the activity of PIP as BE inhibitor in *Mycobacterium tuberculosis (Mtb*) by accumulation of ethidium bromide (EtBr) assay. Firstly, the MIC of EtBr was determined by the Resazurin Microtite Assay Plate (REMA). The EtBr accumulation assay was carried out in *Mtb* $H_{37}Rv$ (ATCC 27294), previously cultured in Middlebrook 7H9-OADC. Then, the culture was rinsed with phosphate buffered saline (PBS, pH 7.4) and the OD_{600} was adjusted to 0.4. After, 0.25 mg/mL EtBr (0.5 x MIC) plus 62,5 mg/mL PIP (0.5 x MIC) were added to the bacterial suspension. Verapamil (VP) was used as control in all assays. Relative fluorescence to EtBr-loaded cells was determined in a VICTOR2 D fluorometer (PerkinElmer, Santa Clara, CA, USA) in 530/25 nm as the excitation wave lengths and 590/20 nm as the detection wave lengths, respectively. The relative fluorescence values were obtained by normalizing the data against the EtBr fluorescence background. The 0.25 mg/mL (0.5 x MIC) EtBr did not affected cell viability (influx-efflux in equilibrium) in *Mtb* $H_{37}Rv$. EtBr efflux was inhibited by VP and PIP. The relative final fluorescence (RFF) was 0,853 for VP and 0,649 for PIP. The results showed that PIP is an efflux pump inhibitor as well as VP in *Mtb.* Additional studies should be performed to better understand PIP action so that it can become an adjunctive drug in the treatment of TB.

Keywords: effluxpump, effluxpumpinhibitor, ethidiumbromide, piperine, tuberculosis.