

**Title:** Piperine modulatory effect in rifampicin activity by inhibition of efflux pumps in *Mycobacterium tuberculosis*

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**Abstract:**

*Mycobacterium tuberculosis* (*Mtb*) is the causative agent of tuberculosis (TB), one of global health problem with the highest mortality and morbidity rates in the world. Considering the high incidence and resistance rates of TB, the development of new treatment strategies to control the disease is necessary. Piperine (PIP), the main alkaloid of *Piper nigrum*, is reported in the literature for its immunomodulatory, antitumor, analgesic and antimicrobial properties, as well as efflux pumps (EPs) inhibitor in *Mtb* and other bacteria. Efflux pumps are membrane-associated active transporters that promote the extrusion of toxic compounds, including antibiotics, from the cell. The aim of this study was to evaluate the gene expression of *Mtb* BEs after exposure to rifampicin (RIF), PIP and RIF+PIP combination. Two *Mtb* clinical isolates (one RIF resistant and one susceptible) were selected for the study. The bacilli were exposed to RIF, PIP and RIF+PIP 0.5 MIC for 24 h at 37 °C, followed by RNA extraction, purification, DNase treatment and synthesis of the first strand of cDNA. The study of the effect of single drugs and in combination on 12 EPs genes was performed by RT-qPCR. The RIF-resistant isolate showed higher level of EPs regulation after exposure to RIF, compared to the susceptible isolate. Regarding exposure to RIF + PIP was observed a significant drop in expression rates comparing RIF to RIF + PIP for all the bacilli studied in several of the 12 assayed genes, except for the *Rv1410c* in 47S isolate. Besides that, there was downregulation of most EPs genes compared to single RIF exposure in two clinical isolates. PIP was able to significantly induce EPs downregulation genes in RIF susceptible and resistant *Mtb*.

**Keywords:** *Mycobacterium tuberculosis*, Piperine, efflux pumps

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