TITLE: Hydrazone H2faihz: application in antituberculosis therapy and toxicological evaluation

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ABSTRACT:
Mycobacterium tuberculosis, the pathogenic agent of tuberculosis (TB) is a serious infectious disease that has caused more deaths worldwide than any other single infectious disease, killing more than 1.5 million people each year; equating to 4,100 deaths a day. Thus, there is an urgent need for newer agents with efficacy against drug-resistant strains and with faster-acting mechanisms. Previous results from our group, showed that hydrazones had promising minimal inhibitory concentration (MIC) with values comparable to or better than those commonly used to treat TB. Therefore, this work aimed to evaluate the antibacterial potential and possible toxicological risks of hydrazone H2faihz. For this purpose, bacterial inhibition and cytotoxicity potentials were assessed using the resazurin microtiter assay (REMA). The resazurin assay uses an indicator dye to measure oxidation-reduction reactions. Reduction related to growth causes the redox indicator to change from oxidized (nonfluorescent, blue) form to reduced (fluorescent, red) form. The fluorescent or colorimetric signal generated is proportional to the number of living cells in the sample.The mutagenicity and genotoxic potential were assessed using the *Salmonella typhimurium* reverse mutation assay (also known as Ames test) and through comet assay (single-cell gel electrophoresis) measuring DNA strand breaks in Balb/c mices. The acute toxicity and bioavailability of the compound were also evaluated *in vivo*. The substance showed anti-mycobacterial activity at a minimal inhibitory concentration (MIC) of 0.346 µg mL $^{-1}$. Non-cytotoxic effects were observed in HaCat and HepG2 cells with an expressive selectivity index (SI) ($SI=IC_{50}/MIC$) of 2167 and 578, respectively. Furthermore, the data demonstrated that H2faihz did not produced any genotoxic effects *in vitro* (Ames test) or *in vivo* (Comet assay). *In vivo*, the substance was readily bioavailable orally with 88% level of bacterial inhibition after only 30 minutes treatment. H2faihz; also, did not demonstrated any visible toxic effects during the acute toxicity test with LD_{50} value considerably higher than Isoniazid, used as reference drug (H2faihz: 1000 mg kg^{-1}; Isoniazid. 300 mg kg^{-1}). In conclusion, our results suggest that H2faihz; is a promising and suitable candidate in anti-TB therapy.

Keywords: tuberculosis, drugs, toxicology, AMES, comet assay

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