Immunomodulatory and intracellular antimycobacterial activity of *Oxyanthus speciosus*

investigated using human (U937) and mouse (RAW 264.7) macrophage cell lines

Introduction

Tuberculosis (TB) has become a global health problem with one-third of the world's population latently infected with the *Mycobacterium tuberculosis* pathogen and 1 in 10 developing active disease in their lifetime. Due to population growth, the incidence of TB is increasing annually and has become a disease of global concern due to the upsurge of HIV/AIDS and resistant strains, thereby resulting in a continued health crisis and financial burden in various parts of the world, especially Asia and Africa. Medicinal plants are used in many parts of southern Africa to treat TB-related symptoms including chest pain, fever and coughing. One such species is *Oxyanthus speciosus* (Rubiaceae), which was selected for further study after promising *in vitro* antimycobacterial efficacy against a range of saprophytic and pathogenic mycobacterial species was reported (Aro et al., 2015).

Methods

The immunomodulatory efficacy of the acetone extract of *Oxyanthus speciosus* leaf extracts against LPS-stimulated U937 macrophages was determined using a cytometric bead array (CBA) flow cytometry technique. The human Th1/Th2 kit comprising a mixture of six cytokines (BD Biosciences) was used (Labuschagné et al., 2013).

The intracellular efficacy of the extract against *Mycobacterium*-infected RAW 264.7 mouse macrophages was also investigated (method modified from Labuschagné et al., 2013). Cells were infected with *M. fortuitum* to a multiplicity of infection, or MOI, of 1:10 (cells:bacilli) and exposed to several concentrations of extract and the positive control rifampicin (0.25X, 0.5X, 1X, and 2X MICs) in triplicate. After 2, 4 and 6 days post-infection, cells were rinsed, lysed and plated on 7H10 agar to determine CFU/ml compared to the controls.

Results and Discussion

The acetone extract of *Oxyanthus speciosus* increased the expression of IL-2 at 100 µg/mL while rifampicin suppressed the expression of this pro-inflammatory cytokine (Figure 1). The *O. speciosus* extract inhibited the stimulation of IL-4 and IL-5, but markedly enhanced the production of IL-10 despite the fact that it also had a good stimulatory effect on IL-2. This indicated a mixed Th1/Th2 effect. The increase in IL-10 is noteworthy as IL-10 is an important regulatory cytokine, preventing excessive inflammation that may be caused by a Th1 response.

The extract was not cytotoxic to RAW 264.7 macrophages at the highest concentration (1 mg/mL) tested. On day 6 post-infection, the intracellular antimycobacterial activity of the *O. speciosus* crude acetone extract at 1X to 4X minimum inhibitory concentration (MIC) was superior to that of rifampicin, with more than 90% reduction in colony forming units. Figure 2 shows the bactericidal activity of the acetone extract of *O. speciosus* (A) and rifampicin (B).

Figure 1: Release of cytokines following treatment of LPS-stimulated U937 macrophages with acetone extract of *Oxyanthus speciosus* and rifampicin

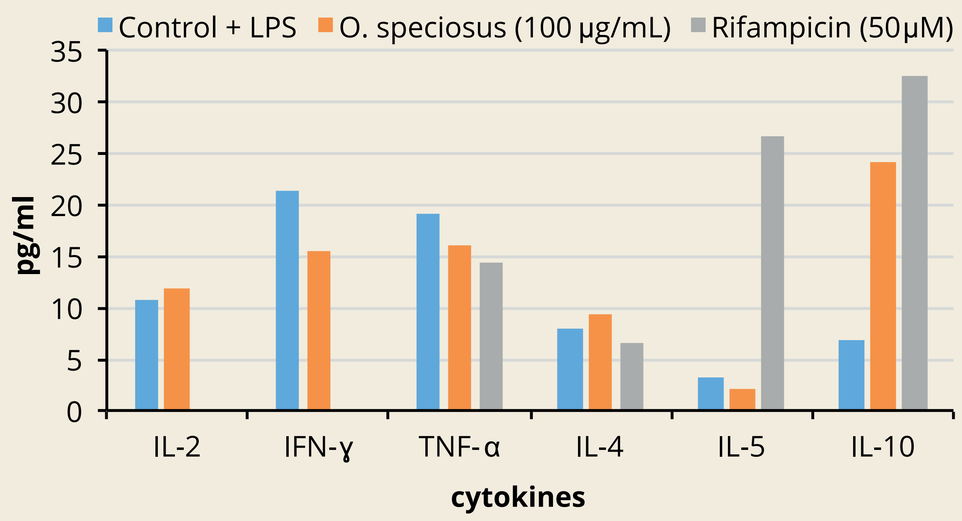
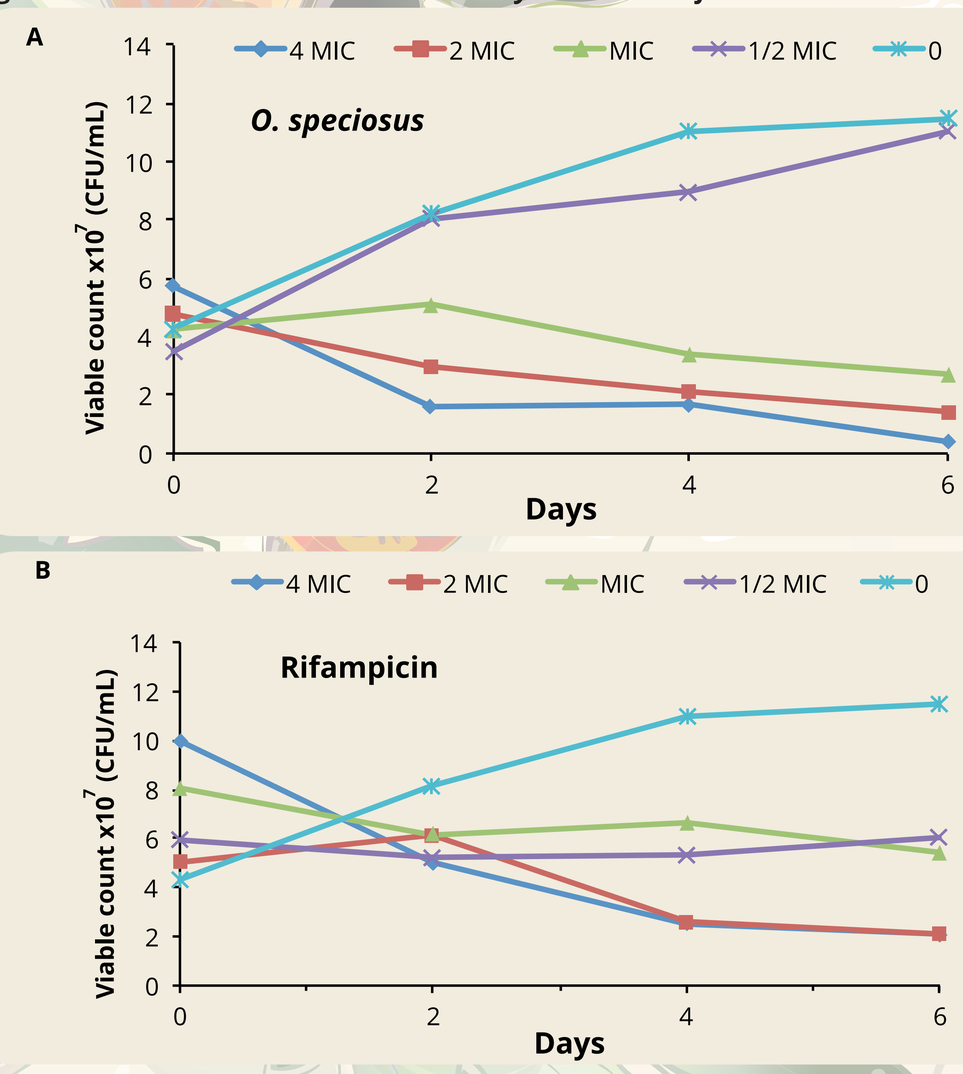


Figure 2: RAW 264.7 cells infected with *Mycobacterium fortuitum*



Conclusion

The extract of *O. speciosus* had a mixed Th1/Th2 effect. Production of Th1 cytokines promotes a protective response to *M. tuberculosis*, but a complex balance of cytokine release from Th1 and Th2 cells is necessary to control infection. Therefore, closer investigation is warranted into the potential immune modulatory activity of *O. speciosus*.

The intracellular bactericidal activity observed was both dose- and time-dependent. The *O. speciosus* acetone extract had good intracellular killing activity, comparable to that of rifampicin. The promising activity of the crude extract of *O. speciosus*, both *in vitro* and intracellularly within macrophages, suggests its potential for use as an anti-TB herbal medicine. This study also supports the use of non-pathogenic mycobacteria as a model for intracellular antimycobacterial activity studies, and comparison using an infectious *Mycobacterium* model is the focus of future work.

References

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