The hepatoprotective capacity of South African *Cyclopia intermedia* against selected ROS inducers

Shanika Reddy[1], Trevor Koekemoer[1], Maryna van de Venter[1]

[1]Biochemistry and Microbiology Department, Faculty of Science, Nelson Mandela University, SouthAfrica, 6001

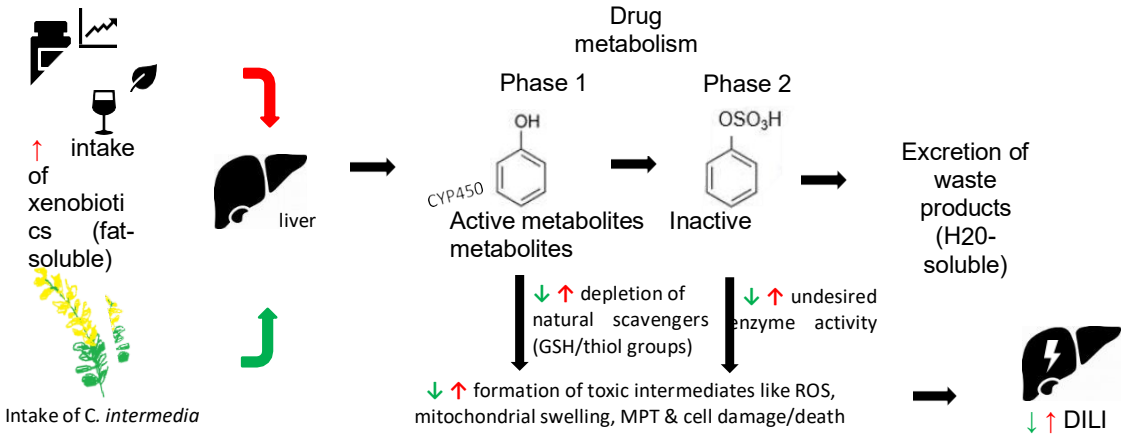

Figure 1: A simplified overview of the events that lead to drug induced liver injury (DILI) and how these adverse effects can be countered through the intake of Cyclopia intermedia during drug metabolism.

Introduction: *Cyclopia intermedia*, commonly known as Honeybush, is endemic to the Cape Fynbos biome and well known for its aroma, pleasant taste as an herbal tea and its use as traditional restorative treatments. *C. intermedia's* acclaimed antioxidant activity, healing and cleansing properties, are as a result of its high flavonoid content, including organic molecules like xanthones. Xanthones are reported to be very stable molecules that play a role in medicinal plant and natural product antioxidant activity; presenting *C. intermedia* as a strong potential therapeutic intervention against reactive oxygen species (ROS) induced pathologies, like drug induced liver toxicity (DILI). The lack of knowledge and desperation for medical relief has aggravated the incidence of DILI, making it a health priority to find innovative and cost-effective ways to properlyscreen and evaluate natural products.

Method: Aqueous *C. intermedia* extract was screened against C3a, CaCo2 and VERO cell lines, using Hoechst 33342-PI dual labelling to rule out formsof cytotoxicity and genotoxicity. To assess hepatoprotective effects, C3a cells were pretreated with *C. intermedia* for 24 hours and then exposed to ROS inducers, like menadione or TBHP. Mode of cell death, cell cycle analysis, oxidative stress, mitochondrial membrane depolarization (ΔΨm), and glutathione reserve were assessed using quantitative fluorescence microscopy and appropriate fluorescent dyes. Antioxidant assays (DPPH, NO, ORAC, CAPe and FRAP) were also used.

Results: *C. intermedia* was both non-cytotoxic and non-genotoxic at all of the tested concentrations. Antioxidant assessments showed that *C. intermedia* decreased both menadione-and TBHP-induced ROS production, protected against menadione-induced ΔΨm depolarization,protected against TBHP-induced changes in thiol group levels, and decreased apoptotic cells in a dose dependent manner. Finally, *C. intermedia* demonstrated high levels of scavenging and suggested moderate bioavailability in the CAPe assay.

Discussion and Conclusion: Together these assays addressed several aspects relating to DILI and hepatoprotection, and served as a valuable evaluation of *C. intermedia's* antioxidant capacity. Further investigation is necessary to confirm whether the extract can effectively reach systemic circulation. *C. intermedia* holds great promise as an hepatoprotectant, but before it can dubbed as a 'safe and more cost effective' treatment against DILI, it is recommended that the *in vitro* data be confirmed *in vivo*.

References: Joubert *et al.,* 2008; Devarbhavi, H., 2012. Alexander, L., 2018.

Keywords: *Cyclopia intermedia,*DILI, antioxidant capacity, hepatoprotection.