***In silico* docking and in vitro studies, identified curcumin, 18α-glycyrrhetinic acid, rosmarinic acid, and quercetin as inhibitors of α-glucosidase and pancreatic α-amylase and lipid accumulation in HepG2 cells – important type 2 diabetes targets**

Kadima Tshiyoyo

University of Pretoria, South Africa

Introduction: Type 2 diabetes (T2D) is a chronic disease characterised by prolonged hyperglycaemia due to the inability of the liver, muscle and fat cells to absorb glucose following insulin stimulation and is often associated with non-alcoholic liver disease (NAFLD). Several therapeutic targets have been identified, including the inhibition of α-amylase and α-glucosidase in the small intestine and the inhibition of hepatic lipid accumulation. The aim of this study to determine the ability of curcumin, 18α- Glycyrrhetinic acid (18α-GA), quercetin and rosmarinic acid to inhibit α-amylase and α-glucosidase activity, to reduce cell viability and to inhibit hepatic lipid accumulation.

Methodology: *In silico* enzyme inhibitory abilities of compounds found in 30 commercially available herbs and spices were assessed using docking analysis with Maestro from Schrodinger and AutoDock vina from DIA-DB; four compounds with docking scores more negative or similar to acarbose were selected for further analysis. In silico ADMET properties were obtained using canvas and the pkCSM online tool, and the results were compared with acarbose. *In vitro* biochemical assays were used to confirm docking studies; DNSA and pNPG assays were used for α-amylase and α-glucosidase inhibition, respectively. The IC_{50} of each compound was determined with the sulforhodamine B assay in the C2C12, HepG2 and Caco-2 cell lines, representing muscle, liver and intestinal tissue respectively. The ability to reduce lipid accumulation in the oleic acid (OA) HepG2 hepatic model for NAFLD was evaluated. For enzyme inhibition and lipid accumulation studies, known drugs acarbose and metformin respectively were used as controls.

Results: The relationships between *in silico* and *in vitro* inhibition results correlated well; a more negative docking score in silico correlated with a lower inhibition constant (K_i) in vitro. For α-glucosidase, the K_i values of curcumin, 18α-GA, and quercetin were significantly lower ($p < 0.05$) than acarbose with no significant difference ($p > 0.05$) between acarbose and rosmarinic acid. For α-amylase, the K_i values of curcumin, 18α-GA, quercetin, and rosmarinic acid were significantly higher ($p < 0.05$) than acarbose. A IC_{50} was determined for 18α-GA, quercetin and rosmarinic acid in C2C12, 18α-GA and rosmarinic acid in HepG2 and curcumin and rosmarinic acid in Caco-2 cells. At the concentrations used to evaluate OA induced lipid accumulation, the compounds were not cytotoxic. All compounds and metformin significantly reduced ($p < 0.05$) OA induced lipid accumulation in HepG2 cells.

Discussion and conclusion: Curcumin, 18α-GA, quercetin, and rosmarinic acid inhibited α-glucosidase, and reduced the accumulation of OA-induced droplets in HepG2 cells, indicating that plant derived compounds can potentially inhibit glucose uptake and reduce hepatic lipid accumulation often associated with T2D. Herbs and spices are rich source of these compounds providing a cost-effective, easily cultivated, and readily available source of compounds that can alleviate T2D symptoms. Curcumin is found abundantly in turmeric and rosmarinic acid in rosemary and peppermint, where a dose of 1.3 g of turmeric or 1.6 g of peppermint or rosemary is equivalent to 50 mg acarbose per meal.

References: (1) Pereira, A. S., Banegas-Luna, A. J., Peña-García, J., Pérez-Sánchez, H. & Apostolides, Z. 2019. Evaluation of the anti-Diabetic activity of some common herbs and spices: providing new insights with inverse virtual screening. Molecules, 24(22), p.4030-4071
(2) Tolmie, M., Bester, M. J. & Apostolides, Z. 2021. Inhibition of α-glucosidase and α-amylase by herbal compounds for the treatment of type 2 diabetes: A validation of in silico reverse docking with in vitro enzyme assays. Journal of Diabetes, 13(10), p.779-791

Keywords: α-amylase, α-glucosidase, herbal compounds, reverse molecular docking, type 2 diabetes