Modulation of inflammation and apoptosis in intestinal cells by aqueous rooibos extracts – an assessment of the effect of rooibos polyphenols on gut health

Shana de Bruyn[1], Stefan Abel[1] and Mariska Lilly[1]

Cape Peninsula University of Technology, Bellville, South Africa.

Introduction: The gastrointestinal (GI) tract is a complex system affecting organism health, thus maintaining integrity thereof is of utmost importance. It has been suggested that polyphenols have the ability to suppress inflammation in gut enterocytes via several mechanisms. *Aspalathus linearis* or rooibos is a popular herbal tea and is traditionally used to treat asthma, colic disorder, allergies and dermatological problems. Furthermore, women in rural areas consume rooibos during pregnancy to relieve indigestion and stomach cramps. Rooibos is known as a natural source of antioxidants with aspalathin as the most abundant flavonoid found in tea extracts. Consequently, the potent *in vitro* antioxidant effects of rooibos extracts make it an attractive choice for therapy for numerous ailments especially for GI related issues.

Aim: This study aims to analyse the anti-inflammatory effects of aqueous unfermented (RgU) and fermented (RgF) rooibos tea extracts on IPEC-J2 intestinal epithelial cells, including cell integrity indices
i.e. cell viability and apoptosis.

Methodology: Aqueous rooibos extracts were prepared and characterized by HPLC analysis and the total phenolic content determined by spectrophotometry. The half-maximal inhibitory concentration (IC50) values of 0.2 mg/ml (RgU) and 0.5 mg/ml (RgF) were determined. Sub-cytotoxic concentrations of 0.1, 0.05 and 0.025 mg/ml for RgU and 0.2, 0.1 and 0.05 mg/ml for RgF were selected for the pre- exposure model. IPEC-J2 cells were pre-exposed to the selected tea concentrations for 24 hrs. Thereafter inflammation was induced using 10 µg/ml *Escherichia coli* lipopolysaccharide (LPS) for an additional 24 hrs.

Results: Both stimulated (+ LPS) and non-stimulated (-LPS) samples displayed cell viability levels above 60 %. However, apoptosis (determined via Caspase 3/7 assay) for +LPS and -LPS conditions were found to be relatively high, with RgF (0.2 mg/ml) inducing the highest apoptotic activity with a 3-fold increase. Anti-inflammatory effects of the aqueous rooibos tea extracts were determined by measuring the inflammatory biomarker IL-8. RgU and RgF displayed anti-inflammatory properties by decreasing IL-8 at all extract concentrations. The RgU at 0.1 mg/ml had the strongest anti-inflammatory effect for both stimulated and unstimulated conditions.

Discussion and Conclusion: In this study the lowest concentration RgU exhibited promising protective effects by displaying strong anti-inflammatory activity against IL-8 and this was associated with minimal cytotoxic effects. In order to get a better understanding of the interaction between rooibos tea and different drugs, the expression and activity of NF-kB, the modulation of ABC transporters as well as the effects on the intestinal barrier integrity will be investigated. These experiments will shed light on the mechanisms of rooibos tea in conjunction with current medication regimes and will provide a better understanding of the protective effects of rooibos tea on intestinal gut health.