Effect of rooibos and red palm oil supplementation, alone or in combination, on cardiac function after exposure to hypertension and inflammation in an ischaemial/reperfusion injury model

Cardiovascular disease (CVD) is without a doubt one of the most challenging health issues of our time and accounts for the highest number of deaths in both developed and developing countries. Despite the huge strides that have been achieved in the diagnosis and therapeutic intervention of CVD, the disease burden still remains enormous. Therefore, this calls for novel and innovative interventions to curb the surge of CVD. The use of plant based food with bioactive phytochemicals, has a great potential to reduce the incidence of CVD, specifically in resource-strained countries. Red palm oil (RPO) and the indigenous herbal tea, rooibos have previously been shown to exhibit potential cardioprotective effects. Their health promoting properties have largely been attributed to their antioxidant and anti-inflammatory activities and emerging evidence also showed that they have the potential to modulate cell signalling events. Substantial scientific evidence proposes oxidative stress and inflammation to play an important role in the pathogenesis of cardiovascular disease. Hence, natural plant extracts such as RPO and rooibos could be recommended as adjuvants to clinical therapy to reduce the morbidity and mortality associated with CVD. This thesis reports on three studies investigating the cardiovascular protective effects that chronic feeding of either RPO, rooibos or their combination have on 1) antioxidant enzymes and the NO-cGMP pathway in myocardial tissue of spontaneous hypertensive rats, 2) the modulation of systemic and myocardial inflammation and 3) the myocardial ischaemic/reperfusion tolerance in a rat model of lipopolysaccharide induced inflammation. The aim of the first study was to investigate the effect of RPO on cardiac function in spontaneously hypertensive rats. The role of the nitric oxide cyclic-guanosine monophosphate (NO-cGMP) pathway, (as determined by the nitric oxide (NOS) activity) and the antioxidant defence system (selected antioxidant enzymes) were also investigated. Cardiac function was monitored at stabilization and reperfusion using the Langendorff perfusion system. Antioxidant enzymes were determined from left ventricular tissue, while total NOS activity was determined in the aorta and left ventricular tissue. The results show that RPO offered cardiac protection as evidenced by improved left ventricular developed pressure (LVDevP), maximum velocity of pressure rise (+dp/dt) max and fall (-dp/dt) max during reperfusion in spontaneously hypertensive rats (SHR) compared to their control counterparts. Improved function in SHR was associated with increased myocardial superoxide dismutase 2 (SOD2) protein expression compared to the normotensive rats. There was differential modulation of the NOS activity by RPO, an increase in NOS activity was observed in the aorta while a reduction in the activity of NOS was observed in the left ventricular tissue of both RPO supplemented normotensive and hypertensive rats compared to their respective control groups. These results argue a role for elevated NO production in the aorta for endothelial function maintenance. Increased SOD2 protein might lead to reduced oxidative stress. Thus, NO-cGMP pathway and antioxidant defense systems synergistically acted to restore cardiovascular function in SHR. The aim of the second study was to investigate the effect of RPO and rooibos supplementation on the modulation of systemic and myocardial inflammation in a rat model. As RPO and rooibos contain different types of antioxidants which reside and exert their biological effects in different cellular compartments, the combination of these two natural food compounds has the potential to enhance the spectrum
of available dietary antioxidants in different cellular compartments, which could result in a better protection against certain pathological conditions such as inflammation. The Langendorff system and the lipopolysaccharide (LPS)-induced inflammatory model were used to determine if RPO and rooibos could protect against the negative effect of LPS-induced inflammation on baseline cardiac function. Both inflammation and dietary supplementation did not have any effect on baseline cardiac functional parameters. Our results show that administration of LPS resulted in elevated plasma levels of IL-1β in supplemented and non-supplemented rats indicating that an inflammatory response was triggered in the LPS-treated rats. However, this increase in IL-1β was counteracted by concurrent elevation of plasma IL-10 in LPS-induced rats consuming either rooibos or RPO alone. Furthermore, the combination of RPO and rooibos enhanced myocardial IL-10 levels in LPS-induced rats. This data shows a difference in response to LPS injection between the myocardium and the systemic circulation. The results indicate that the combination of these two natural food substances exhibit potential anti-inflammatory properties which could be beneficial in clinically relevant conditions where inflammation plays a role. Having shown that dietary intervention with RPO and rooibos had the potential to modulate the inflammatory response in the model of inflammation at basal conditions, we then proceeded to the third study to specifically establish if dietary RPO when supplemented alone will improve functional recovery and reduce infarct size in LPS-treated hearts. The Langendorff perfusion system was employed for determination of cardiac function and infarct size. The roles of NFκB, p38 MAPK and the myocardial antioxidant defence systems were investigated as potential mechanisms of protection. LPS-treatment caused significant increases in myocardial IL-1 β indicating that inflammation was induced. However, the levels of myocardial IL-10 was reduced in LPS-treated hearts compared to the non-treated hearts. Intervention with dietary RPO resulted in improved functional recovery and reduced infarct size, in both healthy hearts and in the LPS-treatment group. The RPO-induced cardio-protection was associated with increases in myocardial protein expression of the antioxidant enzymes, SOD1, SOD2, GPX1 as well as increased p38 phosphorylation during reperfusion. LPS treatment increased myocardial protein expression of NFκB p65 which was reversed by RPO supplementation. Reduction of myocardial NFκB protein expression, increased p38 phosphorylation and elevated mitochondrial antioxidant (SOD2 and GPX1) as well as cytosolic enzymes (SOD 1) are proposed as potential mechanisms underlying the RPO-induced cardio-protection in this model. Based on these study results, for the first time, having included vasculature aspects in the cardio-protective effects of RPO we have shown that the NO-cGMP pathway and antioxidant defense systems may act synergistically to restore cardiovascular function in spontaneously hypertensive rats. Results from the second study also provide the first scientific evidence that RPO in combination with rooibos (a flavonoid rich endemic herbal tea) could have potential anti-inflammatory activities at systemic as well as myocardial level, which may be beneficial in clinically relevant conditions where inflammation plays a role. From the third study it can be concluded that dietary RPO improved myocardial tolerance to ischaemia-reperfusion injury in a model of inflammation.