

REVIEW

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Hyperglycemia-induced oxidative stress and heart disease-cardioprotective effects of rooibos flavonoids and phenylpyruvic acid-2-O- β -D-glucoside

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Abstract

Diabetic patients are at an increased risk of developing heart failure when compared to their non-diabetic counterparts. Accumulative evidence suggests chronic hyperglycemia to be central in the development of myocardial infarction in these patients. At present, there are limited therapies aimed at specifically protecting the diabetic heart at risk from hyperglycemia-induced injury. Oxidative stress, through over production of free radical species, has been hypothesized to alter mitochondrial function and abnormally augment the activity of the NADPH oxidase enzyme system resulting in accelerated myocardial injury within a diabetic state. This has led to a dramatic increase in the exploration of plant-derived materials known to possess antioxidative properties. Several edible plants contain various natural constituents, including polyphenols that may counteract oxidative-induced tissue damage through their modulatory effects of intracellular signaling pathways. Rooibos, an indigenous South African plant, well-known for its use as herbal tea, is increasingly studied for its metabolic benefits. Prospective studies linking diet rich in polyphenols from rooibos to reduced diabetes associated cardiovascular complications have not been extensively assessed. Aspalathin, a flavonoid, and phenylpyruvic acid-2-O- β -D-glucoside, a phenolic precursor, are some of the major compounds found in rooibos that can ameliorate hyperglycemia-induced cardiomyocyte damage in vitro. While the latter has demonstrated potential to protect against cell apoptosis, the proposed mechanism of action of aspalathin is linked to its capacity to enhance the expression of nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*) expression, an intracellular antioxidant response element. Thus, here we review literature on the potential cardioprotective properties of flavonoids and a phenylpropanoic acid found in rooibos against diabetes-induced oxidative injury.

Keywords: Diabetes mellitus, Hyperglycemia, Oxidative stress, Cardiomyopathy, Rooibos, Polyphenols

Background

The prevalence of diabetes mellitus (DM) is increasing at an alarming rate worldwide. According to recent data by the International Diabetes Federation, the number of individuals living with DM is 415 million, and this figure is estimated to reach 642 million by the year 2040 [1]. Type 2 DM, which is associated with insulin resistance and obesity, represents approximately 90% of diabetic

cases worldwide [2]. Type 1 DM is characterized by deficient insulin secretion and chronic hyperglycemia. Chronic hyperglycemia remains the leading causal factor for the development of cardiovascular disease (CVD) and heart failure (HF) in a diabetic state [3, 4]. Chronic hyperglycemia alters the myocardial substrate preference in cardiomyocytes and augments production of free radical species, giving rise to oxidative stress [5]. Oxidative stress may directly induce cardiac structural remodeling, a prominent sign of diabetic cardiomyopathy (DCM) [6, 7]. DCM is a distinct clinical entity that was first described about four decades ago [7]. The diagnosis of DCM remains nebulous and the precise mechanism explaining DCM has been partially explained. Although

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they remain mainly non-ischemic, distinctively affecting the heart muscle, cardiomyopathies play a pre-dominant role in inducing HF and are one of the major causes of death in Africa [8, 9]. Currently, there is no specific treatment for DCM; however, therapeutic drugs certainly play a significant role in the treatment of diabetes and its cardiovascular complications.

Insulin and metformin are the commonly used therapies for the treatment of DM and have been shown to present limited cardiac protective properties [10–12]. It is known that CVD-related deaths in individuals with DM are still a major concern [13]. Furthermore, lifestyle intervention of restricted energy intake and physical activity in persons with impaired glucose tolerance has been shown to improve CVD function. Nonetheless, most individuals do not adhere to such lifestyle interventions. On the other hand, antioxidants are among the leading therapies being investigated for their efficacy against various metabolic complications [14, 15]. In the last decade, there has been much interest in the potential health benefits of plant polyphenols, such as resveratrol, mangiferin and aspalathin as dietary antioxidants [16–18]. The rooibos flavonoid and dihydrochalcone, aspalathin, has been investigated and reported to contribute to the antidiabetic properties of rooibos extract as reviewed by Muller and colleagues [18]. This flavonoid and others have been shown to exert their therapeutic effects by mainly regulating the expression of key genes involved in energy metabolism and oxidative stress. Prime examples include 5'-adenosine monophosphate-activated protein kinase (AMPK), which is crucial for maintenance of myocardial substrate metabolism and nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a transcription factor that is upregulated in response to oxidative stress [18, 19]. This review will discuss the cardioprotective potential of rooibos flavonoids and the phenylpropenoic acid, phenylpyruvic acid-2-O- β -D-glucoside (PPAG), against hyperglycemia-induced injury and heart disease. Physiological context is provided by a short overview of the role of oxidative stress in a diabetic heart.

Mechanisms of oxidative stress leading to cardiac tissue damage

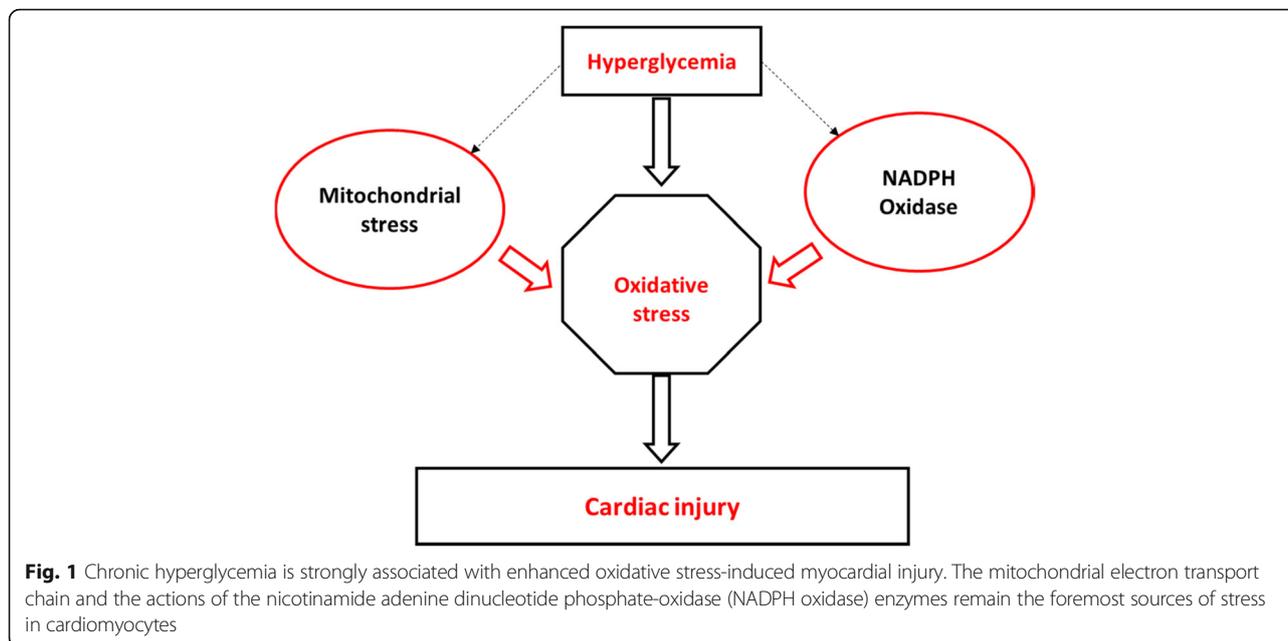
Chronic hyperglycemia is strongly associated with enhanced oxidative stress-induced myocardial injury [13, 20]. This has been confirmed by various laboratory studies showing strong correlation between oxidative stress and matrix remodeling in cardiomyocytes isolated from diabetic heart tissue [21, 22]. Oxidative stress is aggravated by enhanced levels of Reactive Oxygen Species (ROS) within cardiomyocytes [21, 23, 24]. Abnormal ROS production elicits an increased pro-inflammatory response resulting in myocardial apoptosis [25]. Some of the

well-known reactive oxygen substances, associated with myocardial damage include superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). Generation of ROS is generally a cascade of reactions that starts with the formation of O_2^- [26]. Superoxide anion rapidly dismutates to H_2O_2 , either spontaneously or catalytically by superoxide dismutase (SOD), while H_2O_2 is decomposed by catalase (CAT) to water and oxygen. However, the mitochondrial electron transport chain and the actions of the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase; Nox) enzymes remain the foremost sources of stress in cardiomyocytes (Fig. 1). Mitochondrial structural modification is affiliated with reduced endogenous antioxidant status in cardiomyocytes from diabetic heart tissue [24, 27]. Correspondingly, augmented activity of NADPH oxidase is demonstrated in the myocardium of diabetic animals at the same rate as oxidative damage [28].

The mitochondrion is an essential organelle for intracellular energy production. Increasing the cellular demand of the mitochondrion to produce energy is associated with accelerated ROS production. Given that a diabetic heart has a diminished mitochondrial antioxidant capacity [29], it is therefore not surprising that minor alterations in mitochondrial structure or function induced by increased ROS are associated with major changes in the heart muscle [30]. Increased ROS and mitochondrial depolarization, subsequent to diastolic dysfunction, have been reported in patients with metabolic disturbances [31, 32]. However, data explaining the precise role of mitochondrial dysfunction in a diabetic heart are still lacking.

Concurrent with energy generation is the constant generation of ROS within the mitochondria [33]. Accumulation of these radicals results in the induction of mitochondrial permeability transition (MPT) and the opening of high conductance permeability transition pores [34]. MPT formation has been reported to lead to an altered redox state of the mitochondria [30]. With disease states, the MPT opening is unique for being nonselective and allowing for the accumulation of excessive calcium (Ca^{2+}) and other toxic compounds [35, 36]. The fate of the cell after an insult depends on the extent of MPT pore formation [36–38]. If MPT pore formation occurs only to a limited extent, the cell may recover through cell recovery mechanisms such as activation of mitophagy/ubiquitination, whereas if MPT pore formation is exacerbated, it accelerates apoptosis [38]. If it occurs to an even larger degree, the cell is likely to undergo necrotic cell death [38]. Thus, prevention of mitochondrial membrane depolarization may play a role in reducing myocardial injury associated with chronic hyperglycemia.

On the other hand, Nox is another system that plays a notable role in the generation of ROS in many cell types,



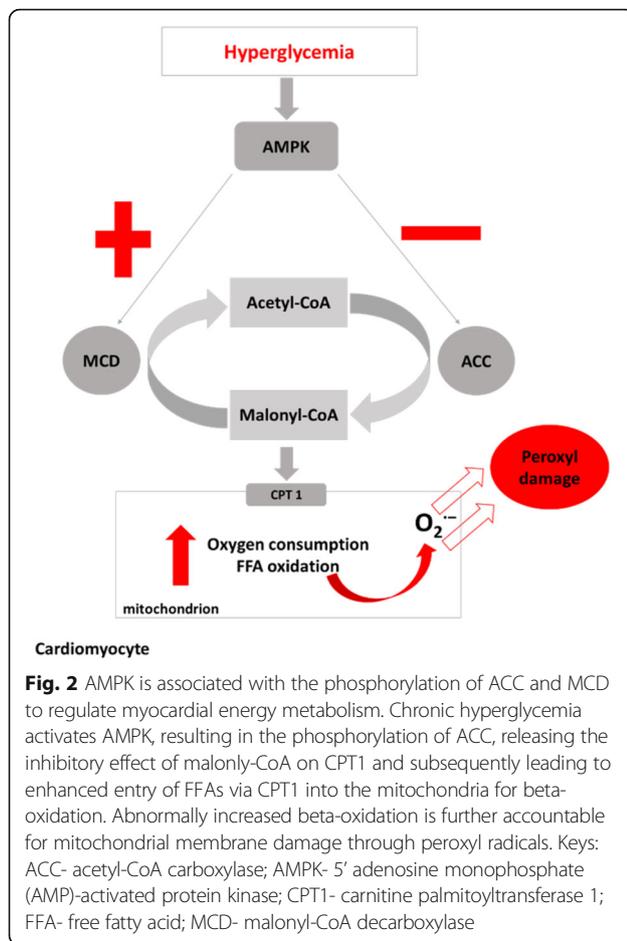
including cardiomyocytes. Nox generates intracellular ROS by transferring electrons from NADPH across the cell membrane and coupling these to molecular oxygen to produce $O_2^{\cdot-}$. Nox exists in different isoforms, i.e. Nox1 to Nox4, with Nox2 and Nox4 predominant in the heart muscle [39, 40]. Nox1 has been identified to be the major source of ROS production in vascular tissues, resulting in low levels of nitric oxide [41, 42]. Decreased levels of nitric oxide are connected to impaired endothelium-dependent vasodilation of coronary arteries [43]. Human aortic endothelial cells exposed to high glucose concentrations display amplified expression of Nox1 concomitant to enhanced oxidative damage [44]. The same study showed that diabetic mice lacking Nox1 had profound anti-atherosclerotic outcome related to reduced ROS generation. Although Nox2 and Nox4 are predominant in the heart muscles, these two ROS producing enzymes are also expressed in other cell types and are implicated in agonist-stimulated redox-sensitive signal transduction [39]. Nox2 has been shown to play a central role in insulin resistance-mediated oxidative damage in vascular tissue [45, 46]. Nox2 knockout transgenic mice with endothelial-specific insulin resistance present reduced ROS production and vascular dysfunction [45]. On the other hand, the Nox4 isoform is specifically expressed in mitochondria of cardiomyocytes; and mice lacking the Nox4 gene show reduced free radical damage [28]. Its overexpression in the mouse heart deteriorates cardiac dysfunction by initiating apoptosis through cytochrome c release [28]. Cytochrome c is a vital component of the mitochondrial electron transport chain, acting as an electron shuttle during redox generation of ATP [47]. Its

release and mitochondrial depolarization are considered key physiological events of apoptosis [48]. In cardiomyocytes exposed to high glucose concentrations, cytochrome c release is enough to cause apoptosis, independent of mitochondrial depolarization status [49]. Complex systems within the apoptotic pathway exacerbate myocardial injury through cytochrome c release [50]. Therefore, interventions that could inhibit pro-apoptotic proteins and mitochondrial cytochrome c release could salvage myocardial injury.

Endogenous cardioprotective mechanisms

Activation of AMPK

AMPK is a heterotrimeric protein composed of a catalytic alpha, non-catalytic beta and gamma subunit. The main function of this kinase is to preserve ATP or to promote alternative pathways of ATP generation. It functions as a sensor during low energy states such as ischemia to change substrate utilization and thereby increase or decrease ATP synthesis. Its activation is controlled by an increase in the AMP:ATP ratio [51]. Stimulation of AMPK leads to phosphorylation of many target proteins important for ATP synthesis and utilization while concurrently inhibiting ATP-consuming pathways such as fatty acid synthesis. In the diabetic heart, AMPK activation is linked to phosphorylation of both acetyl-CoA carboxylase and malonyl-CoA decarboxylase; however, its association with the latter in the heart remains to be fully elucidated [52–54]. Acetyl-CoA carboxylase and malonyl-CoA decarboxylase are both important for the interconversion of acetyl-CoA to malonyl-CoA (Fig. 2). Phosphorylation of acetyl-CoA carboxylase by AMPK



reduces the generation of malonyl-CoA; thus promoting the entry of FFAs for beta-oxidation into mitochondria through carnitine palmitoyltransferase I [55]. Resultant increased levels of ATP and citrate through beta-oxidation are responsible for the allosteric inhibition of glycolysis through phosphofructokinase-1 [56]. This causes accelerated ROS production and associated membrane peroxidation [24, 26]. Hence, adequate control of uptake and oxidation of FFAs remain crucial for optimal functioning of the myocardium, especially in a diabetic state.

Activation of Nrf2

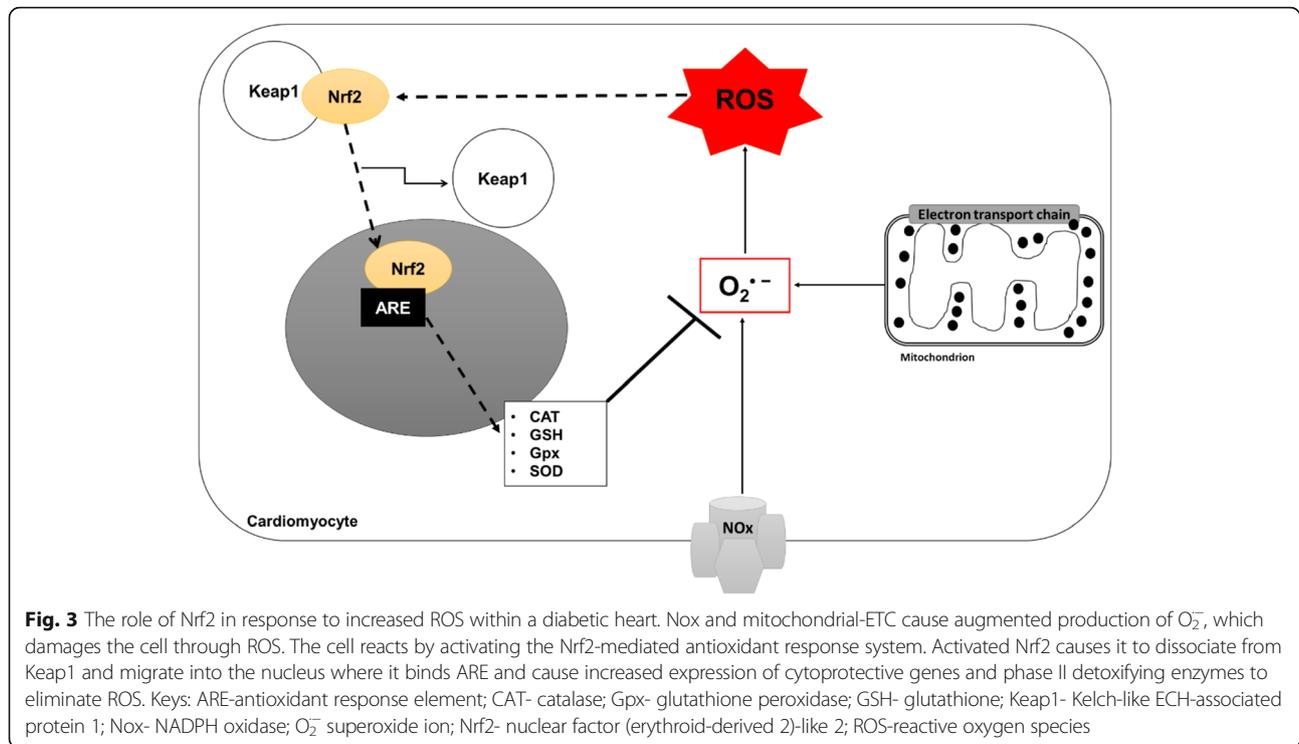
Free radicals within mitochondria are generally removed by mitochondrial SOD, thereby generating H_2O_2 . This process allows H_2O_2 to be further reduced to water by glutathione (GSH) or CAT. GSH remains an important intracellular antioxidant to prevent free radical damage. GSH can easily be oxidized to its disulfide form during oxidation reactions; thus, NADP transhydrogenase enzymes remain important to maintain the reduced form of this co-factor [57]. NADP transhydrogenase functions by transferring electrons from a reduced form of nicotinamide adenine dinucleotide (NADH) to $NADP^+$ in order to

regenerate GSH [58]. Reduced expression of GSH is consistently reported in experimental models investigating a diabetic heart [21, 24]. This was confirmed when investigated in either human subjects at risk of CVD or mice that are chronically subjected to hyperglycemia and hyperlipidemia [59, 60].

Expression of antioxidant response genes, including GSH is regulated by the redox-sensitive transcription factor, Nrf2 [61]. Nrf2 is a transcriptional regulator that is activated in response to intracellular stress (Fig. 3). Genes activated by Nrf2 can be classified into different groups, including phase II detoxifying and cytoprotective enzymes. Nrf2 resides in the cytoplasm, where it is subjected to continuous degradation by the ubiquitin-proteasome [62]. Under stressful conditions such as ischemia or oxidative stress, Nrf2 is activated by disassociating from its negative regulator Kelch-like ECH-associated protein 1 (Keap1) and translocates to the nucleus. Once in the nucleus, it forms a heterodimer with Maf protein before binding to the antioxidant response element (ARE) to initiate and activate antioxidant defence genes [63]. An overview of the pathway associated with the activation of Nrf2 and its protective effect against ROS in a cardiac cell is illustrated by Fig. 3. Activation of Nrf2 in epithelial cells has been shown to induce GSH synthesis and thus protects against oxidative stress [64]. In addition to its negative regulation of Nrf2, Keap1 also acts as a sensor for a wide array of stressors that could activate Nrf2. Significant down-regulation of cardiac Nrf2 expression is concomitant to increased ROS and reactive nitrogen species damage in hearts of diabetic animals [62–64]. Thus, agents that can significantly up-regulate Nrf2 expression have a potential to protect cardiomyocytes against high glucose-induced apoptosis.

Cardioprotective potential of current antidiabetic agents

Primary interventions that may salvage a diabetic heart at risk from myocardial infarction mainly target maintaining low blood sugar levels [13]. While such interventions are achievable and beneficial to the heart, adherence to lifestyle changes remains a big challenge. Therefore, antidiabetic agents that could suppress postprandial and chronic hyperglycemia may be effective in decreasing the risk of HF. Despite evidence on the efficacy of antidiabetic and antidyslipidemic drugs such as dipeptidyl peptidase-4 inhibitors and statins [65, 66], metformin remains the leading first line antidiabetic drug for type 2 diabetic individuals with known cardiac complications [67, 68]. In addition to its accomplished antidiabetic properties [69, 70], metformin is associated with improved clinical outcomes in diabetic patients with HF [71, 72]. Although clinical data are lacking, metformin enhances the efficacy of a number of synthetic drugs and novel medicinal compounds currently screened for metabolic benefits in vitro [73–75]. Metformin monotherapy or its use as an add-on effect to



glibenclamide improves the intracellular antioxidant status of the myocardium in streptozotocin-induced diabetic Sprague-Dawley rats [76]. In addition to improving the antioxidant capacity of heart cells, metformin may benefit the heart by enhancing autophagy and inhibiting MPT opening [77, 78]. However, an increasing toll of cardiovascular related deaths in diabetic patients on treatment warrants further investigation into alternative treatment regimes.

Cardioprotective potential of rooibos

In recent years, the use of plant-derived products as a cardioprotective therapy is receiving increasing attention [59, 79, 80]. Rooibos (*Aspalathus linearis*) is an indigenous South African plant well-known for its potential health benefits. Rooibos tea is available in two forms: a “fermented” or oxidized form; and an “unfermented” or unoxidized form (Fig. 4). The unfermented product is also referred to as green rooibos. The “fermentation” process gives fermented rooibos its distinctive reddish-brown colour, while unfermented rooibos tea maintains its green colour (Fig. 4b). The fermentation process is important to develop the characteristic taste and aroma of rooibos tea, traditionally consumed [81]. Its effect on the health outcomes of rooibos is obscured if the products do not originate from the same bush due to large inherent variation in the phenolic content of the rooibos plant [81]. However, it is well established fermentation decreased the flavonoid content of rooibos [81]. Infusions and extracts prepared from unfermented rooibos

have higher antioxidant capacity than those from fermented rooibos, largely due to higher levels of flavonoids, in particular aspalathin in the unoxidized plant material [81]. Consumption of a “ready-to-drink” unfermented rooibos beverage as opposed to one produced from fermented rooibos effected a 28% higher total radical-trapping antioxidant potential in the plasma of human subjects [82]. Nonetheless, both forms of rooibos are increasingly studied in experimental diabetes and related complications, given that fermented rooibos is more readily available and forms the bulk of rooibos production [81].

In a recent study, Oh and colleagues have demonstrated that the total flavonoid content of a water extract of rooibos, determined using a colorimetric assay based on aluminium complexation, is higher than that of lemongrass tea, mulberry leaf tea, bamboo leaf tea, lotus leaf tea, and persimmon leaf tea [83]. However, they further showed that the total flavonoid content of this extract is slightly lower than that of green and black tea. Von Gadow and colleagues have also previously shown that both fermented and unfermented rooibos, when tested together with green, oolong and black tea, present strong antioxidant properties in vitro [84]. They further showed that this antioxidant effect, as evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical assay, was reduced in the order: green tea > unfermented rooibos > fermented rooibos > semifermented rooibos > black tea > oolong tea. Accompanying its well-documented

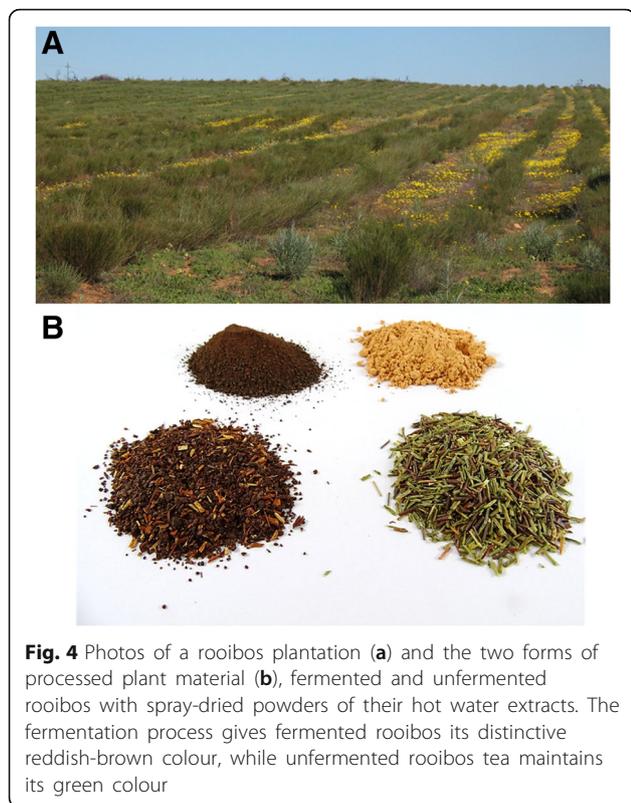


Fig. 4 Photos of a rooibos plantation (a) and the two forms of processed plant material (b), fermented and unfermented rooibos with spray-dried powders of their hot water extracts. The fermentation process gives fermented rooibos its distinctive reddish-brown colour, while unfermented rooibos tea maintains its green colour

antioxidant capacity [18, 84–89], rooibos has been shown to inhibit adipogenesis in vitro [90], reverse palmitate-induced insulin resistance in 3T3-L1 adipocytes [91] and prevent inflammation in vivo [92–94]. An aspalathin-enriched green rooibos extract, containing 18.4% aspalathin has demonstrated an even higher hypoglycemic potential through its inhibitory effect of alpha-glucosidase and suppressing fasting plasma glucose levels in animal models [91, 95]. Another green rooibos extract, containing 6.6% aspalathin promoted glucose transporter 4 translocation to the plasma membrane and suppressed advanced glycation end products (AGEs)-induced oxidative damage in cultured skeletal L6 muscle cells, pancreatic beta-cells and obese diabetic KK-Ay mice [96]. Rooibos inhibits experimentally-induced oxidative stress [21]; and improved cardiovascular function by reducing lipid peroxidation, blood pressure and angiotensin-converting (ACE) enzyme in various experimental models [97–100]. Moreover, it protected against ischemia-reperfusion injury by modulating the phosphatidylinositol 3-kinase/protein kinase B (PI3K-AKT) pathway [101]. Controlling free fatty acid oxidation by modulating phosphorylation of AMPK remains central in the preventive effect of rooibos against diabetes associated cardiac complications [90, 91, 96]. The cardioprotective potential of rooibos is summarized in Table 1.

A profound relationship between a diet rich in polyphenols and health has given hope to long-term effective interventions that could prolong the onset of diabetes and its co-morbidities [102]. Flavonoids constitute a major sub-class of polyphenols, which can be further divided into different sub-groups such as dihydrochalcones, flavonols and flavones, the predominant rooibos flavonoid sub-groups. Variations in the hydroxylation pattern, glycosylation and chromane ring (Ring C) underpin their structural differences (Table 2). Structural features of flavonoids relevant for their antioxidant properties [103] also explain binding affinity to plasma proteins [104] and enzymes such as alpha-glucosidase [105]. Whilst in vitro data suggest flavonoid aglycones to be more effective than their glycosides, lack of in vivo data precludes broad generalizations concerning the effect of glycosylation on the benefits of flavonoids for human health [106].

In the following sections the focus will fall on flavonoids found in rooibos that have an ameliorative effect against various metabolic diseases. Of interest is the predominance of C-glucosyl flavonoids in rooibos, and in particular the dihydrochalcones (aspalathin and nothofagin) and their flavone derivatives (orientin, isoorientin, vitexin and isovitexin). The major flavonols are O-glycosyl derivatives of quercetin, i.e. quercetin-3-O-robinobioside, hyperoside, isoquercitrin, and rutin. The aglycones, luteolin, chrysoeriol and quercetin, are present in low to trace levels in rooibos [107]. Discussion of their bioactivities is included to underscore the health potential of rooibos.

PPAG is per definition not a phenolic compound due to the absence of a hydroxyl group on the phenyl ring. This compound is a biosynthetic precursor of flavonoids [108] and rooibos is one of the few plants demonstrated to date to be a substantial source.

Rooibos dihydrochalcones

The dihydrochalcone, aspalathin is unique to rooibos, while its 3-deoxy analogue, nothofagin, is relatively rare with its presence also confirmed in *Notofagus fusca* and *Schoepfia chinensis* [81]. Unfermented rooibos tea beverage contains 10-fold or more aspalathin and nothofagin when compared to the fermented product [107, 109]. This is not surprising as the fermentation process is known to reduce their content in rooibos [81, 110]. Despite C-glycosides having very low bioavailability due to the inability of intestinal enzymes to hydrolyze the C-C bond linking the sugar moiety to the aglycone and thus influencing their absorption process [111], aspalathin has been reported in the plasma of subjects who consumed 500 mL of green rooibos infusion, containing 287 mg aspalathin [112]. However, generally as reported for other dihydrochalcones [113, 114], the human gut

Table 1 The cardioprotective effect of rooibos, its flavonoids and a phenylpropenoic acid

Rooibos/compounds	Model	Experimental outcome	References
Rooibos	Aqueous extract of fermented rooibos on cardiomyocytes isolated from diabetic rats	Prevented experimentally induced oxidative stress and ischemia	[21]
	Fermented rooibos tea for 6 weeks in human subjects at risk of cardiovascular disease (CVD)	Reduced CVD risk by improving lipid profile and redox status	[59]
	Aqueous extract of fermented rooibos in endothelial cells from human umbilical veins (HUVECs)	Prevented vascular-induced inflammation by enhancing nitric oxide production	[86, 92, 93]
	Aqueous extract of fermented rooibos on non-diabetic rats	Acted as a bronchodilator, antispasmodic and blood pressure lowering effects	[97]
	Fermented rooibos tea in healthy human subjects	Prevented myocardial infarction by inhibiting angiotensin-converting enzyme (ACE)	[98–100]
	Aqueous extracts of fermented and unfermented rooibos in non-diabetic rats	Reversed ischemia-reperfusion injury	[101]
Aspalathin and nothofagin	Aspalathin and nothofagin on high glucose-induced vascular in HUVECs and mice	Prevented inflammation and thrombosis by suppressing TNF- α , IL-6 and NF- κ B	[118, 119]
	Aspalathin in H9c2 cardiomyocytes exposed to high glucose and cardiomyocytes isolated from insulin resistant rats	Prevented cell apoptosis by reducing phosphorylation of AMPK; decreasing inflammation and lipid accumulation; and attenuated oxidative damage via increasing <i>Nrf2</i> expression	[73, 124–126]
Orientin and isoorientin	Orientin on isolated hearts of nondiabetic rats, rabbits and guinea pigs as well as H9c2 cells	Prevented ischemia-reperfusion injury and platelet aggregation by inhibiting mPTP formation and apoptosis	[137–139]
Orientin and isoorientin	Orientin on rats	Prevented myocardial infarction	[134]
	Isoorientin in low density lipoprotein isolated from human plasma	Prevented formation of atherosclerotic lesions by inhibiting low density lipoprotein (LDL) oxidation	[135]
	Orientin in non-diabetic rats	Attenuated ventricular remodeling associated with myocardial infarction	[136]
	Orientin and isoorientin in lipopolysaccharide-induced reperfusion injury	Protected vascular barrier integrity by inhibiting hyperpermeability	[223]
Vitexin and isovitexin	Vitexin on primary cardiomyocytes and isolated rat hearts and on rats	Prevented ischemia-reperfusion injury by reducing calcium overload and modulating ERK1/2 signaling and MAPK pathway	[146, 149, 224]
	Vitexin on primary rat cardiomyocytes	Prevented cardiac hypertrophy by inhibiting calcineurin and CaMKII signaling pathways	[151]
	Vitexin on dogs	Reduced aortic pressure, arterial and pulmonary capillary pressure and heart rate	[150, 225]
	Vitexin on rats	Attenuated acute doxorubicin cardiotoxicity by reducing oxidative stress and apoptosis	[226]
Luteolin and chrysoeriol	Luteolin on isolated rat cardiomyocytes, rabbit hearts and anesthetized pigs	Prevented ischemia-reperfusion injury and enhanced relative coronary flow	[157, 159, 162]
	Luteolin on rat endothelium-denuded aortic rings	Induced vasorelaxation by regulating calcium and potassium channels and reducing oxidative stress	[227]

Table 1 The cardioprotective effect of rooibos, its flavonoids and a phenylpropenoic acid (*Continued*)

	Luteolin on vascular smooth muscle cells and rats	Prevented hypertensive vascular remodeling	[160]
	Luteolin on diabetic and normal rats	Alleviated vascular complications associated with insulin resistance through the Ppar α pathway	[161]
Luteolin and chrysoeriol	Luteolin-7-glucoside on isolated primary rat cardiomyocytes	Prevented ischemia-reperfusion injury and increased of coronary flow	[228]
	Chrysoeriol in rats under anesthesia and H9c2 cells	Reduced arterial blood pressure and protected against doxorubicin-induced cardiotoxicity	[97, 172]
Quercetin and rutin	Quercetin on rats	Protected against diabetic cardiomyopathy, autoimmune myocarditis, LDL-oxidation, and doxorubicin-induced lipid peroxidation	[185–192]
	Quercetin in either endothelial cells or rats	Presented antihypertensive potential and reduced cardiac hypertrophy by increasing antioxidant capacity	[229–233]
Hyperoside and rutin	Hyperoside in vitro and in vivo	Protected against hyperglycemia induced inflammation	[208]
	Hyperoside in ECV304 cells	Prevented advanced glycation end products and promoted via the c-Jun N-terminal kinases (JNK) pathway	[205]
	Hyperoxide in vitro and in vivo	Hydrogen peroxide induced cell damage and ischemia reperfusion injury	[209, 211, 212]
	Rutin on rats	Protected against advanced glycation end products, oxidative stress and myocardial infarction	[199, 234, 235]
Phenylpyruvic acid-2-O- β -D-glucoside (PPAG)	PPAG on high-glucose exposed H9c2 cells	Protected against substrate impairment, mitochondrial depolarization and cell apoptosis	[221]

microbiota can possibly enhance the absorption of aspalathin and nothofagin in the small intestine by splitting off the aglycone from the glucose moiety [115]. In vivo, the low levels of aspalathin are difficult to detect in serum, however metabolites (glucuronides and sulfates) of aspalathin and nothofagin have been detected in urine of human subjects 5 h after consumption of 500 mL of either fermented or unfermented rooibos [109]. Recent data have demonstrated that aspalathin is absorbed and metabolized in mice to mostly sulphate conjugates detected in urine, while the mode of absorption is hypothesized to occur through the monolayer paracellularly [116].

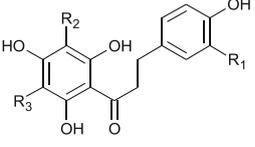
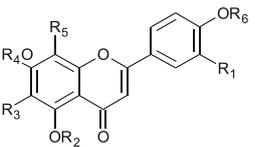
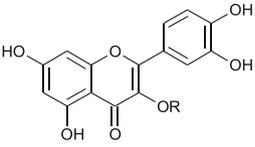
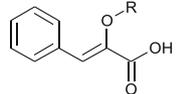
The biological activity of aspalathin and nothofagin has been primarily associated with their known strong antioxidant properties [73, 117]. Both compounds protected against high glucose-induced vascular inflammation and platelet aggregation when tested in endothelial cells and mice; however, nothofagin did not have any anticoagulant effect in mice [118, 119]. Increasing research is presented focusing on aspalathin and its enhanced efficacy to prevent metabolic-associated complications in vitro and in vivo models [18, 120–122]. Our laboratory has presented recent evidence that

aspalathin reversed palmitate-induced insulin resistance in cultured adipocytes [123], while it prevented high glucose-induced apoptosis by improving substrate metabolism in H9c2 cells exposed to high glucose or cardiomyocytes from insulin resistant rats [73, 124, 125]. In addition to regulating AMPK and enhancing *Nrf2* expression, aspalathin can modulate the expression of peroxisome proliferator-activated receptor gamma and sterol regulatory element-binding protein 1/2, transcriptional factors involved in lipid metabolism, in addition to inhibiting inflammation via interleukin-6/Janus kinase 2 pathways, leading to reduced myocardial apoptosis [73, 124, 126].

Rooibos flavones

The major flavones present in rooibos include orientin and isoorientin, the flavone derivatives of aspalathin, and vitexin and isovitexin, the flavone derivatives of nothofagin. Minor flavones include the aglycones, luteolin, and chrysoeriol (Table 2). Lower levels of flavones are present in fermented rooibos [81]. Food processing may also change their content. The orientin and isoorientin content of a ready-to-drink rooibos beverage showed a slight change as a result of pasteurization and storage,

Table 2 Molecular structures of flavonoids and a phenylpropenoid present in rooibos

Structures	Names
Dihydrochalcones	
	Aspalathin: R ₁ = OH; R ₂ = H; R ₃ = β-D-glucopyranosyl Nothofagin: R ₁ , R ₂ = H; R ₃ = β-D-glucopyranosyl
Flavones	
	Luteolin: R ₁ = OH; R ₂ , R ₃ , R ₄ , R ₅ , R ₆ = H
	Chrysoeriol: R ₁ = OH; R ₂ , R ₃ , R ₄ , R ₅ = H; R ₆ = CH ₃ Orientin: R ₁ = OH; R ₂ , R ₃ , R ₄ , R ₆ = H; R ₅ = β-D-glucopyranosyl Isoorientin: R ₁ = OH; R ₂ , R ₄ , R ₅ , R ₆ = H; R ₃ = β-D-glucopyranosyl Vitexin: R ₁ , R ₂ , R ₃ , R ₄ , R ₆ = H; R ₅ = β-D-glucopyranosyl Isovitexin: R ₁ , R ₂ , R ₄ , R ₅ , R ₆ = H; R ₃ = β-D-glucopyranosyl
Flavonols	
	Quercetin-3-O-robinobioside: R = α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl Isoquercitrin: R = β-D-glucopyranosyl Rutin: R = α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl Hyperoside: R = β-D-galactopyranosyl
Phenylpropenoid	
	Phenylpyruvic acid-2-O-glucoside: R = β-D-glucopyranosyl

postulated to be due to the conversion of aspalathin to these compounds [110, 127]. Except for luteolin, there is very limited data on the characteristic metabolism and transportation of these flavone glucosides. The absorption of orientin, isoorientin and vitexin has been reported on Caco-2 cell monolayers [128, 129], with transporter mediated efflux in addition to passive diffusion shown to be the predominant mode of transportation. In a pharmacokinetics study using Sprague–Dawley rats, intravenous administration of a 20 mg/kg dose of orientin was found to be highly recovered in plasma and eliminated within 90 min after intravenous administration [130]. On the other hand, permeability and absorption rate of luteolin has been shown to be significantly greater in the colon and ileum compared to the duodenum and jejunum in rats [131]. Furthermore, some of these compounds, including isoorientin may be

deglycosylated to their aglycones by gut microbiota as reviewed by Muller et al. [18].

The strong antioxidant properties of flavones have been associated with their free radical scavenging properties [132]. Although very few studies are available on the antidiabetic properties of orientin and isoorientin, extracts with abundant levels of vitexin, orientin and isoorientin have been shown to inhibit adipogenesis in 3T3-L1 adipocytes [90, 133]. Relevant to the cardiovascular system, these compounds have been reported to inhibit high glucose-induced vascular inflammation [134], atherosclerosis [135], cardiac remodeling [136] and ischemia-reperfusion injury [137–139]. Additional protective effects of these compounds are summarized in Table 1 and are mainly mediated by nuclear factor kappa B, a transcriptional factor involved in diabetic-induced HF [140]. Moreover, isoorientin has been shown

to reduce other diabetic associated complications such as lipid toxicity and insulin resistance [141]. Together with direct radical scavenging activity, the protective mechanism of isoorientin has been linked to the induction of Nrf2 pathway-driven antioxidant response through phosphatidylinositol 3-kinase signaling [142]. On the other hand, oral administration of vitexin and isovitexin at 1 mg/kg has been shown to reduce postprandial blood glucose levels in sucrose loaded normoglycemic mice [143]. In addition to inhibiting α -glucosidase [144], vitexin and isovitexin rich extracts have been demonstrated to attenuate diabetes linked complications such as adipogenesis and AGEs in vitro [132, 145]. Vitexin reverses ischemia-reperfusion injury in perfused rat hearts and brain by attenuating inflammatory response and apoptosis [146–149]; it increased coronary artery blood flow and cardiac output in anesthetized animals [150]; and it improved cardiac hypertrophy by reducing the expression of calcium downstream effectors, calcineurin-NFATc3 and phosphorylated calmodulin kinase II (CaMKII), both in vitro and in vivo [151]. Other associated cardioprotective mechanisms of vitexin may include inhibiting cardiomyocyte apoptosis by reducing calcium overload and extracellular signal regulated kinase (ERK1/2) [152].

Furthermore, although available in trace levels in rooibos, release of luteolin from orientin and isoorientin in the gut may enhance the levels to physiological relevance. Accumulative evidence suggests strong ameliorative effect of luteolin against diabetes and CVD associated complications [153–155]. The antidiabetic properties of luteolin include improving hepatic insulin sensitivity by suppressing gluconeogenesis in diet-induced obese mice [153]; it prevented neuronal injury and cognitive performance by attenuating oxidative stress in rats [154]; and attenuated morphological destruction of the kidney in rats [155]. The anti-inflammatory properties of luteolin include inhibiting elevated levels of interleukin-1 β and nuclear factor kappa B [156–158]. Relevant to the heart, luteolin reduced systolic and diastolic blood pressure of various animal models [159, 160]; it improved contractile function [161]; and blocked apoptosis following ischemia-reperfusion in adult rat cardiomyocytes via downregulating microRNA-208b-3p [162]; it attenuated HF in a rat model of DCM [163]; and protected against acute and chronic periods of isoproterenol-induced myocardial infarction by suppressing mitochondrial lipid peroxidation [164]. One of the important mechanisms linked to the cardioprotective effect of luteolin during ischemia-reperfusion injury include regulation of ERK1/2 and c-Jun N-terminal kinase (JNK), which are pathways implicated in generation of inflammation [165]. The number of functional hydroxyl groups on the structure of luteolin directly correlates to its scavenging effect of hydroxyl radicals [166].

Another flavone of interest that is present in very low quantities in rooibos is chrysoeriol [167]. Chrysoeriol has been previously shown to be more effective in the protection against lipid peroxidation than its glycoside (chrysoeriol-6-*O*-acetyl-4'- β -D-glucoside) when tested in vitro [168]. In addition to preventing H₂O₂-induced oxidative stress in osteoblasts [169], chrysoeriol protected Raw264.7 macrophages from lipopolysaccharide-induced inflammation by blocking activator protein 1, which is crucial in the transcriptional activation of inducible nitric oxide synthase [170]. A hydroalcoholic extract of *Tecoma stans*, containing 96% chrysoeriol presented an enhanced activity to inhibit pancreatic lipase [171]. Relevant to the heart, chrysoeriol can lower arterial blood pressure in rats under anesthesia [97]; and it can protect against doxorubicin-induced cardiotoxicity by inhibiting apoptosis in H9c2 cells [172]. However, no published study is available on the effect of chrysoeriol on a diabetic heart at present.

Rooibos flavonols

Quercetin and its glycosides, quercetin-3-*O*-robinobioside, hyperoside, isoquercitrin, and rutin are classified by a distinct 3-hydroxyflavone backbone and are the major flavonols present in rooibos (Table 2). Generally, based on a specific population assessed, the average intake of flavonols may range between 20 to 35 mg/day [173, 174]. Although additional studies are required to validate their bioavailability, flavonol aglycones have been shown to be highly absorbed in the gut [175]. The type of sugar moiety and stability of aglycone largely affect the absorption of each compound as shown for quercetin glycosides from onions being better absorbed than pure aglycones [173, 176]. Another study has demonstrated that isoquercitrin and hyperoside are highly absorbable in rats [177]. Regular consumption of flavonols has been found to be protective against ischemic heart disease in some individuals [178]. Quercetin attenuated paracetamol-induced liver damage and impairment of kidney function such as intracytoplasmic vacuolization and brush border loss in rats [179]. Quercetin has a high affinity to inhibit AGEs such as methylglyoxal and glyoxal in a bovine serum albumin system [180].

Quercetin and rutin further exhibit a broad range of pharmacological activities within the myocardium (Table 1). These compounds presented atherosclerosis lowering properties by reducing hepatic fatty acid synthesis in mice [181]. They enhanced glucose uptake in muscle cells subjected to oxidative stress [182] and prevented against dyslipidemia associated complications such as inflammation and lipid toxicity by enhancing antioxidant capacity in rats [183]. Interestingly, in the heart, quercetin and rutin have been shown to directly alleviate DCM by improving myocardial ultrastructure in diabetic animals through aldose reductase,

oxidative stress inhibitory activity and modulation of cardiac calcium homeostasis [184–187]. The use of quercetin at a dose of 10 mg/kg body weight for 28 days protected against autoimmune myocarditis by suppressing oxidative stress in rats [188]. Quercetin is thought to exert cardiac protection through quenching lipid peroxidation, as it is a known scavenger of peroxy radicals [189–192]. In a double-blind randomized clinical trial on women ($n = 72$), quercetin supplementation (500 mg capsule daily) for 10 weeks significantly reduced systolic blood pressure but had no effect on other cardiovascular parameters and inflammatory biomarkers [193]. Likewise, rutin protected against myocardial damage in a diabetic state by decreasing postprandial hyperglycemia and slowing down formation of AGEs in various experimental models [194–197]. In combination with aspalathin, rutin reduced blood glucose concentrations of streptozotocin-induced diabetic rats over a 6 h monitoring period [95]. It further improved glucose homeostasis in streptozotocin-induced diabetic rats suppressing gluconeogenesis [197]. Regulation of glucose metabolism and increasing intracellular antioxidant capacity have been proposed to be the main cardioprotective effects of both quercetin and rutin [198, 199].

Like quercetin and chrysoeriol, hyperoside is often present in very low quantities in a cup of fermented rooibos tea [167]. Plants and extracts rich in hyperoside have been established to display antidiabetic properties [200–202]. Hyperoside prevented against diabetic nephropathy by inhibiting apoptosis and albuminuria in glomerular podocytes isolated from diabetic rats and mice [203, 204]. Other biological activities of hyperoside include reducing accelerated production of AGEs in ECV304 cells via the JNK pathway [205]; suppression of inflammation through reducing nuclear factor- κ B activation in mouse peritoneal macrophages [206]; and inhibition of α -glucosidase and apoptosis in liver cells [207]. In the heart, hyperoside protected hyperglycemia-induced inflammation *in vitro* and *in vivo* [208]; hydrogen peroxide-induced oxidative damage [209, 210]; and it protected against ischemic-reperfusion injury in isolated rat hearts [211]. The protective mechanism of hyperoside against diabetes and heart associated complications has been mainly through suppressing cell apoptosis, improving mitochondrial function and regulating Nrf2 and extracellular signal-regulated protein kinase signaling [212, 213].

PPAG

PPAG is a phenylpropanoic glucoside (Table 2) that acts as a precursor in the flavonoid biosynthesis pathway and has been shown by various studies to be present in rooibos [18, 81, 108]. The occurrence of PPAG in rooibos was described for the first time about two decades ago [108] and its bioavailability profile is yet to be established. Phenylpyruvic acids apparently play a key role in

the biosynthesis of a number of secondary metabolites, including PPAG [214]. The biological activity of a compound with similar structure to PPAG such as 3-phenylpyruvate has long been reported to display antidiabetic properties [215, 216]. Exposure of cardiomyocytes isolated from diabetic rats to a low concentration of fermented rooibos that contains a relatively high level of PPAG (0.71 g/100 g extract) prevents oxidative stress and apoptosis [21]. Recent findings indicated that this compound attenuates insulin resistance and protects beta cells from obese and streptozotocin-induced mice against endoplasmic reticulum stress-induced apoptosis [217–219]. Data available on the cardioprotective properties of PPAG are limited to a study in H9c2 cardiomyocytes, showing that PPAG abolishes high glucose-induced altered myocardial substrate metabolism and apoptosis by increasing the Bcl2/Bax ratio and reducing caspase 3/7 activity [21]. This study further showed that PPAG displayed reduced capacity to protect H9c2 cells against oxidative stress. This result was anticipated since PPAG is not expected to be an active antioxidant as it lacks the phenolic structural features that are required for free radical scavenging ability [220]. Interestingly, PPAG used in combination with a known antidiabetic agent such as metformin demonstrated better protection of cardiomyocytes exposed to high glucose-induced oxidative stress than when used as a monotherapy [221]. Correspondingly, Patel et al. [222] recently showed that PPAG has no inhibitory effect on cytochrome P450 enzymes, CYP2C8, CYP2C9, and CYP3A4, which are important in the metabolism of hypoglycemic drugs, such as thiazolidinediones and sulfonylureas. Supporting the potential use of nutraceutical agents such as PPAG, especially in combination with a current antidiabetic agent to attenuate oxidative stress-induced damage and protect diabetic individuals at risk of myocardial infarction needs further investigation.

Conclusions

Blood glucose lowering therapies such as metformin and insulin have played a major role in prolonging lives of diabetic patients. However, tight control of blood glucose remains a challenge in such patients. By contrast, ameliorative therapies for oxidative stress, including polyphenols as an adjunct to current blood lowering drugs, show promise in protecting diabetic hearts in experimental models. In recent years, rooibos has gained popularity due to its potential use as a dietary supplement that is rich in polyphenols. The presence of constituents such as aspalathin, nothofagin and PPAG that are unique to rooibos or rarely occur in other plants make it attractive for scientific investigation. The compounds present in rooibos continue to present robust biological properties that are associated with ameliorative effects on inflammation and apoptosis, leading to improved

cardiac function in different animal models. In addition, current evidence has suggested that the combinational use of some of these compounds with known antidiabetic agents such as metformin may enhance their biological efficacy. However, this review clearly highlights the evidence gap pertaining to the molecular mechanisms associated with the cardioprotective effect of rooibos and its polyphenols. Once these molecular mechanisms are established, in addition to verification of such findings in clinical studies, it could make a significant step in accelerating development of an evidenced-based rooibos nutraceutical. It is therefore imperative that we further investigate the mechanism(s) by which rooibos flavonoids and PPAG modulate diabetes-induced cardiovascular related complications thereby identifying new therapeutic candidates.

Abbreviations

ACE: Angiotensin-converting enzyme; AGEs: Advanced glycation end products; AMPK: 5'-adenosine monophosphate-activated protein kinase; ARE: Antioxidant response element; CaMKII: Calmodulin kinase II; CAT: Catalase; CVD: Cardiovascular disease; DCM: Diabetic cardiomyopathy; DM: Diabetes mellitus; ERK1/2: Extracellular signal-regulated protein kinases 1 and 2; FFAs: Free fatty acids; GSH: Glutathione; H₂O₂: Hydrogen peroxide; HF: Heart failure; HUVECs: Human umbilical vein endothelial cells; IL-6: Interleukin-6; JNK- c: Jun N-terminal kinases; Keap1: Kelch-like ECH-associated protein 1; LDL: Low density lipoprotein; MAPK: Mitogen-activated protein kinases; MPT: Mitochondrial permeability transition; NF-κB: Nuclear factor kappa B; Nox: NADPH oxidase; Nrf2: Nuclear factor (erythroid-derived 2)-like 2; PPAG: Phenylpyruvic acid-2-O-β-D-glucoside; ROS: Reactive oxygen species; SOD: Superoxide dismutase

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Authors' contributions

PVD, EJ, CJF, JL and RJ performed literature search and wrote the manuscript. All authors reviewed, edited and approved the final manuscript.

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