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REVIEW



Betanin as a multipath oxidative stress and inflammation modulator: a beetroot pigment with protective effects on cardiovascular disease pathogenesis

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ABSTRACT

Oxidative stress is a common physiopathological condition enrolled in risk factors for cardiovascular diseases. Individuals in such a redox imbalance *status* present endothelial dysfunctions and inflammation, reaching the onset of heart disease. Phytochemicals are able to attenuate the main mechanisms of oxidative stress and inflammation and should be considered as supportive therapies to manage risk factors for cardiovascular diseases. Beetroot (*Beta vulgaris* L.) is a rich source of bioactive compounds, including betanin (betanidin-5-O- β -glucoside), a pigment displaying the potential to alleviate oxidative stress and inflammation, as previously demonstrated in preclinical trials. Betanin resists gastrointestinal digestion, is absorbed by the epithelial cells of intestinal mucosa and reaches the plasma in its active form. Betanin displays free-radical scavenger ability through hydrogen or electron donation, preserving lipid structures and LDL particles while inducing the transcription of antioxidant genes through the nuclear factor erythroid-2-related factor 2 and, simultaneously, suppressing the pro-inflammatory nuclear factor kappa-B pathways. This review discusses the anti-radical and gene regulatory cardioprotective activities of betanin in the pathophysiology of endothelial damage and atherogenesis, the main conditions for cardiovascular disease. In addition, betanin influences on these multipath cellular signals and aiding in reducing cardiovascular disorders is proposed.

KEYWORDS

Free-radical scavenger; gene-modulator; inflammatory suppressor; LDL protection; atherogenesis prevention



Introduction

Cardiovascular diseases (CVD) comprise a class of disorders including peripheral arterial disease, coronary heart and cerebrovascular disease, which block the blood supply to cardiac muscle and the brain due to aggregates formed by inflammatory and immune cells, platelets, oxidized lipids and smooth muscle cells that proliferate in response to vascular endothelial injury (Celermajer et al. 2012; WHO 2017).

Hypertension, hyperlipidemia, diabetes and obesity are pathological conditions considered risk factors for CVD, as they precede the onset of vascular disease when aggravated (Drummond et al. 2011). Furthermore, risk factors for developing CVD may reduce the detoxification capacity of the antioxidant defense system and stimulate excessive reactive oxygen (ROS) and nitrogen (RNS) species production (Drummond et al. 2011; Newsholme et al. 2016). Multiple pathways are enrolled in the establishment of oxidative stress, by direct oxidation mediated by reactive species and downregulation of the activity and expression of genes

related to redox homeostasis, as well as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, superoxide dismutase, catalase and glutathione peroxidases. This pathophysiological condition is called oxidative stress, and results in damage to lipids, proteins, DNA molecules and cell membranes, while also decreasing the availability of nitric oxide (NO) and triggering early events in the pathogenesis of CVD and many other chronic-degenerative diseases (Zhang, Xing, et al. 2015).

Clinical trials and epidemiological evidence have demonstrated the protective effect of the intake of vegetables enriched in antioxidants on oxidative stress and CVD (Zhang, Xing, et al. 2015; Pollock 2016). Many of these bioactive phytochemicals may be involved in maintaining a redox state in the organism due to their scavenging ROS and RNS abilities, while others may either modulate the expression of genes encoding proteins involved in intracellular defense against oxidative damage, compete for active sites in enzymes or bind to receptor sites as antagonists in various subcellular structures (Zielińska-Przyjemska et al. 2012; Esatbeyoglu et al. 2014; Vidal et al. 2014).

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Beetroot (*Beta vulgaris* L.) belongs to the Chenopodiaceae family and is considered as source of important bioactive compounds, including betalains, nitrate (NO_3^-) and phenolic compounds (Baião, Silva, et al. 2017; Baião, de Freitas, et al. 2017). Among betalains, betanin (betanidin 5-O- β -D-glucoside) is the major phytochemical representative, a water-soluble nitrogenated heterocyclic compound that confers the red-violet color to beetroot (Silva et al. 2016). Furthermore, betanin is described as a bioactive compound capable of inhibiting lipid membranes and low-density lipoprotein (LDL) peroxidations, modulating ROS generation and gene expression in order to reduce inflammatory cytokine release and increasing antioxidant enzyme activities (Esatbeyoglu et al. 2015). Some of the biological effects exhibited by betanin are underlied in two redox-sensitive pathways, the nuclear factor kappa B (NF- κ B) and the nuclear factor erythroid-2-related factor – antioxidant response element (Nrf2-ARE), the main gene transcribers for inflammatory and detoxifying/antioxidant responses (Fiordelisi et al. 2019; Satta et al. 2017). Thus, betanin displays potential as a supportive therapy used to ameliorate the pathophysiological effects caused by the oxidative stress and inflammatory events that lead to CVD diseases.

In this context, this review aims to discuss the origin and the relationship of the betanin structure and its free-radical scavenger activity. The effects of betanin on oxidative stress and pro-inflammatory cascade will be reviewed to indicate the ways in which this compound can reduce the risk factors for CVD development. In addition, the putative molecular mechanisms by which betanin can exert their pharmacological cardioprotective effects will also be discussed. We hypothesize that betanin may protect endothelial function by acting as an inflammatory cascade supressor and/or oxidative homeostasis effector by modulating molecular signaling pathways.

Betanin biosynthesis plasticity and bioaccessibility

Betalains (or chromoalkaloids) are natural water-soluble pigments obtained from the plants of 10 families belonging to the Caryophyllales order that accumulate these compounds in flowers, fruits and occasionally in tissues, although they may also be found in fungi species associated with plants (Delgado-Vargas, Jiménez and Paredes-López, 2000). Betalains are synthesized from the amino acid tyrosine in the shikimate pathway. First, tyrosine is hydroxylated to form 3,4-dihydroxy-L-phenylalanine (L-DOPA) which, in turn, is converted to betalamic acid [4-(2-oxoethylidene)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid] in a two-step reaction initiated by the enzyme DOPA 4,5-dioxygenase (Steglich and Strack 1990; Girod and Zryd 1991; Khan and Giridhar 2015). Alternatively, L-DOPA is oxidized and cyclized to cyclo-DOPA [cyclo-L- (3,4-dihydroxyphenylalanine)], which spontaneously condenses with betalamic acid, forming red pigments, betacyanins, with betanidin as the common structure to all (Kobayashi et al. 2001; Butera et al. 2002). Betalamic acid can also condense with amino acids or amino derivatives forming yellow pigments, betaxanthins (Schliemann, Kobayashi, and Strack 1999; Khan and Giridhar 2015) (Figure 1).

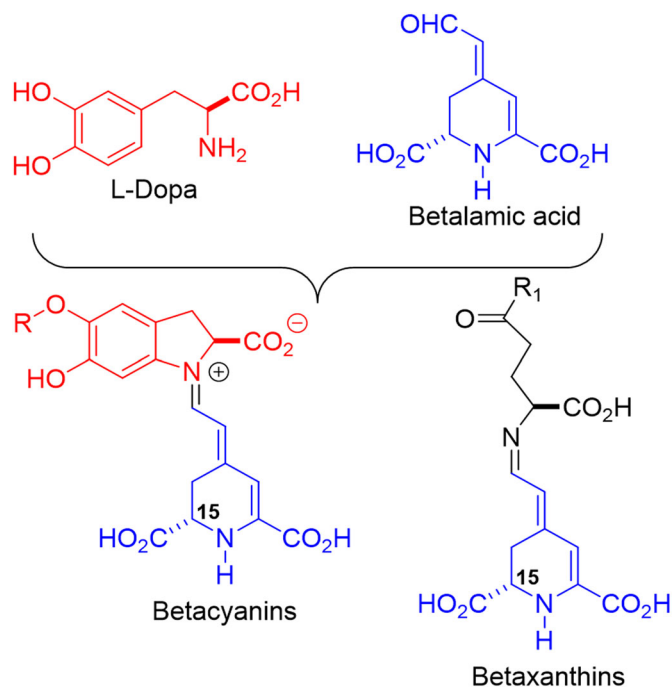


Figure 1. General structures of betaxanthins and betacyanins, and their biosynthetic precursors.

Glycosylation and acylation in the hydroxyls located at the C-5 or C-6 positions, occur, giving rise to several betacyanins, such as prebetanin, isobetanin, neobetanin and betanin (betanidin 5-O- β -D-glucoside), present in purple pitaya (*Hylocereus polyrhizus*) and, mainly, in red beetroot (*Beta vulgaris*) (Supplementary material 1) (Delgado-Vargas, Jiménez, and Paredes-López 2000; Herbach, Stintzing, and Carle 2004).

Betanin obtained from beetroot has several industrial applications. As it occurs naturally in edible plants, it is considered nontoxic to human beings and has been approved by Food and Drug Administration (FDA) and European Union as a dye at *quantum satis* under E162 code (EFSA 2015; FDA, 2009). Betanin corresponds to approximately 75–95% of betalains in red beetroot, ranging from 3.0 to 7.6 mg·g⁻¹ of dry matter. Farming management and post-harvesting conditions can affect betanin content, but it is by far the major pigment compared to isobetanin (1.2–3.1 mg·g⁻¹ of dry matter), betanidin (0.8–1.0 mg·g⁻¹ dry matter) and betaxanthin (2.71–4.25 mg·g⁻¹ dry matter) (Kujala et al. 2002; Slatnar et al. 2015; Sawicki, Baczek and Wiczowski 2016).

Betanin color is due to their conjugated structure, which can lead to resonance and electronic transitions, although the resonance phenomena confers intrinsic structural instability. This is an important aspect to be considered, since this instability affects color during food preparation, processing and storage (Slimen, Najar and Abderrabba 2017; Khan and Giridhar 2015). The optimum pH for betanin ranges from 4.0 to 6.0, and, when outside this range, oxidation causes marked changes in color, such as pigment or food product darkening (Elbe, Maing, and Amundson 1974). The presence of metals like Cu (II) and Fe (III) also decreases betanin stability (Attoe and von Elbe 1984; Czapski 1990; Wybraniec et al. 2013).

Heat treatment of beetroot juice for 30 min at 85 °C causes racemization of the chiral 15S-15R carbon of betanin,

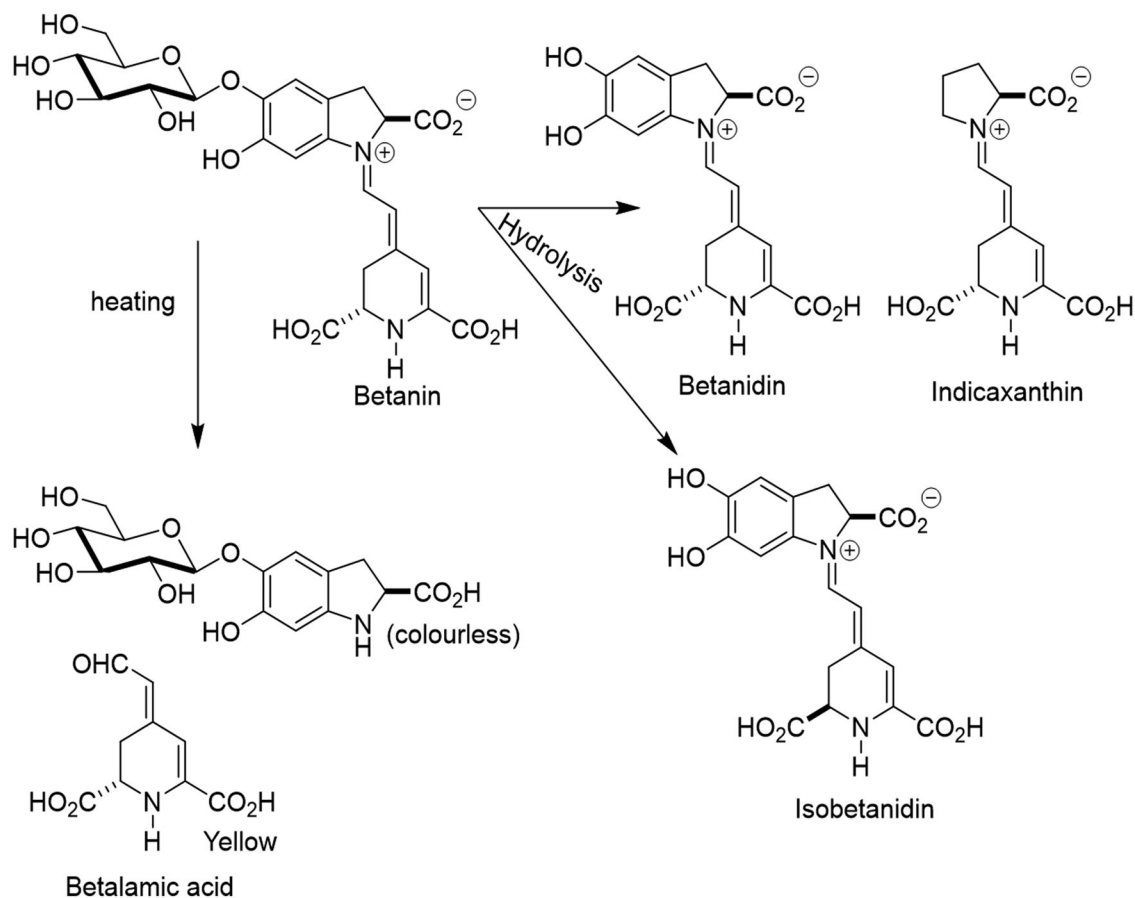


Figure 2. Betanin decomposition by heating and hydrolysis.

resulting in color changes by the formation of yellow betalamic acid and a colorless fragment, cyclodopa 5-O- β -glucoside. The acid-catalyzed hydrolysis of the glycosidic bond depends on pH and other physical-chemical parameters (i.e. light, presence or absence of oxygen, metals and other chemicals) and generates betanidin and its enantiomer, isobetanidin (Figure 2).

Betanin maintains its stability over a wide pH range, from 3 to 7, with variable degradation below pH 2 or above pH 9 (Polturak and Aharoni 2018). Isomerization, decarboxylation or cleavage reactions may occur during thermal or acidic processing, where only cleavage is accompanied by total loss of color (Castellanos-Santiago and Yahia 2008; Stintzing, Schieber, and Carle 2000). Furthermore, during food processing, betanin, as well as other compounds with phenolic structures, may suffer from the action of endogenous enzymes, such as the beta-glycosidases, polyphenol oxidases and/or peroxidases that may contribute to decolorization following pigment decompartmentation, resulting in red color losses and browning (Stintzing and Carle, 2004). However, betanin exhibits the ability to continuously degrade and regenerate during storage (Silva, Baião, et al. 2019). Regeneration occurs through partial *de novo* synthesis from its hydrolytic products, L-DOPA-5-O-glycoside and betalamic acid, rapidly generating betanin when precursor compounds are mixed in solution. Therefore, the maintenance of processed food containing betanin at pH < 5 and temperature < 10 °C can regenerate

betanin, compensating for coloring losses (Huang and Von Elbe 1987).

Betanin has been applied to refrigerated and frozen foods such as sorbets, dairy products and meats (i.e. sausage) as a natural food coloring (EFSA 2015), often replacing anthocyanins, due to its higher water solubility and stability in pH ranging from 3.5 to 7. Betanin is suitable for coloring acidic food items, such as fermented foods, as well as neutral ones, matching optimal conditions for pigment stability (Polturak and Aharoni 2018). Furthermore, betanin is used as a dye in cosmetics and pharmaceuticals products, which range from neutral to low alkaline pH (Esatbeyoglu et al. 2015; Blaak and Staib 2018). In addition to its use as a food coloring additive, betanin is considered a natural food preservative superseding synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), due to its ability to avoid deterioration of oxidation-susceptible foods (Sucu and Turp 2018; Silva, Baião, et al. 2019).

Betanin has demonstrated high stability during storage under freezing and refrigeration temperatures at pH 7.0 and when added to chilled meat samples. Betanin remains biologically active and capable of inhibiting lipid peroxidation during the shelf life of these chilled products (Silva, Baião, et al. 2019). In the gastrointestinal tract, around 50% of oral administered betanin resists stomach gastric acid and remains stable after intestinal digestion, at 37 °C (Tesoriere et al. 2008; Silva, Baião, et al. 2019). When assayed in Caco-

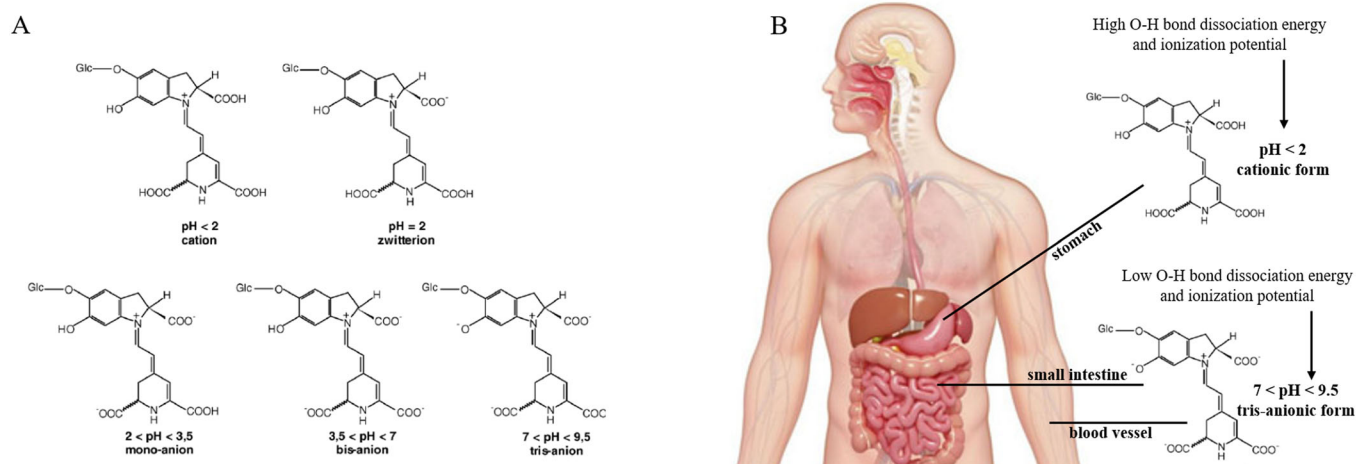


Figure 3. Structural betanin modifications in the gastrointestinal tract and bloodstream. Panel A: Ionic states of betanin at a pH range from 2 to 9. Panel B: Probable ionic states of betanin, phenolic O-H homolytic bond dissociation energy and ionization potential energy according to the pH of gastrointestinal tract fluids and the bloodstream.

2 cells, 65% of betanin crossed the intestinal epithelial cells monolayer by passive diffusion, maintaining its chemical structure during trans-epithelial transport, indicating that it can reach the bloodstream in its active form (Tesoriere et al. 2013). Betanin, primarily used as a food additive, shows bioaccessibility and may be considered a bioactive phytochemical compound when in body fluids, since pH and temperature extremes should not occur.

Relationship between betanin structure and its free-radical scavenger ability

Regarding the ability to remove reactive oxygen or nitrogen species, the antioxidant power of betanin lies in the hydroxyl group of the phenol moiety and/or the cyclic amine group, similar to the phenolic antioxidant ethoxyquin, which are good hydrogen donors and confer reducing properties to this class of compounds (Kanner, Harel, and Granit 2001; Gliszczynska-Swiglo, Szymusiak, and Malinowska 2006).

The pH of different fluids in the human body undergoes variations, influencing betanin stability and bioactivity in the digestive tract compartments. Salivary fluid has a pH of about 7.4, whereas stomach fluid is maintained between 1.5 and 3.5. The abdominal cavities, including the small and large intestines, display pH of 7.4, and finally the blood, with a pH close to 7.4 (Jin 2007). Physiological pH fluids induce changes in the chemical structure of betanin along the systemic routes. Betanin presents a cationic form at pH < 2, zwitterionic form at pH 2, anionic mono at 2 < pH < 3 with deprotonated C2-COOH and C15-COOH groups, anionic bis at 3.5 < pH < 7 with C2-COOH, C15-COOH and C17-COOH deprotonated groups and anionic tris at 7.5 < pH < 9 with all carboxyl deprotonated groups, in addition to the C6-OH on the phenolic ring (Figure 3) (Frank et al. 2005).

The free-radical scavenger activity of betanin at different pH (from 2 to 9) was previously determined through the TEAC assay, phenolic O-H homolytic bond dissociation energy (OH BDE), ionization potential (IP) and deprotonation energy (DE). The TEAC assay confirmed that the

antioxidant activity of betanin is pH-dependent, higher above pH 4. Moreover, the gradual deprotonation of the betanin molecule (mono, bi- and tri-deprotonated) occurs with increasing pH, where BDE and PI decrease significantly (Gliszczynska-Swiglo, Szymusiak, and Malinowska 2006).

Furthermore, the antioxidant power of betanin was demonstrated by assaying a 23 mg·mL⁻¹ aliquot in physicochemical conditions mimicking those found in the small intestinal environment, even after exposure to an acidic suspension similar to gastric fluid. Betanin was able to inhibit over 90% of OH·-radical production in the total antioxidant potential (TAP) assay. In the ferric-reducing ability of plasma (FRAP) assay, betanin was effective in donating electrons and reducing reactive species, such as the ferric ion in the tripyridyltriazine complex (Fe³⁺-TPTZ), to its ferrous state (Fe²⁺-TPTZ), reaching more than 1000 µmol Fe²⁺·L⁻¹. Furthermore, betanin was effective in reducing the ABTS⁺ radical, as measured in the trolox equivalent antioxidant capacity (TEAC) and oxygen radical antioxidant capacity (ORAC) assays, achieving more than 4000 and 2000 µmol Trolox·L⁻¹, respectively. The high antioxidant activity of betanin is due to its greater ability to donate H⁺ and electrons associated with reduced energy for the dissociation of OH bonds and ionization potential, when transforming from the cationic to the mono, bis and/or tri deprotonated states with increasing pH along the gastrointestinal tract (Silva, Baião, et al. 2019). This means that, when betanin is absorbed into the bloodstream, it will remain at the ideal pH to exert its free-radicals scavenger activity (Figure 3).

Betanin, atherogenesis and cardiovascular diseases

Inappropriate lifestyle and eating habits, like diets rich in saturated and *trans* fats and simple sugars, associated with toxins from smoking, alcohol, stress, a sedentary lifestyle and aging, lead to the development of comorbidities such as obesity, diabetes mellitus, dyslipidemia (hypercholesterolemia, hypertriglyceridemia and mixed hyperlipidemia) and arterial hypertension (WHO 2003). Evidence has shown that the aforementioned physiopathological conditions favor

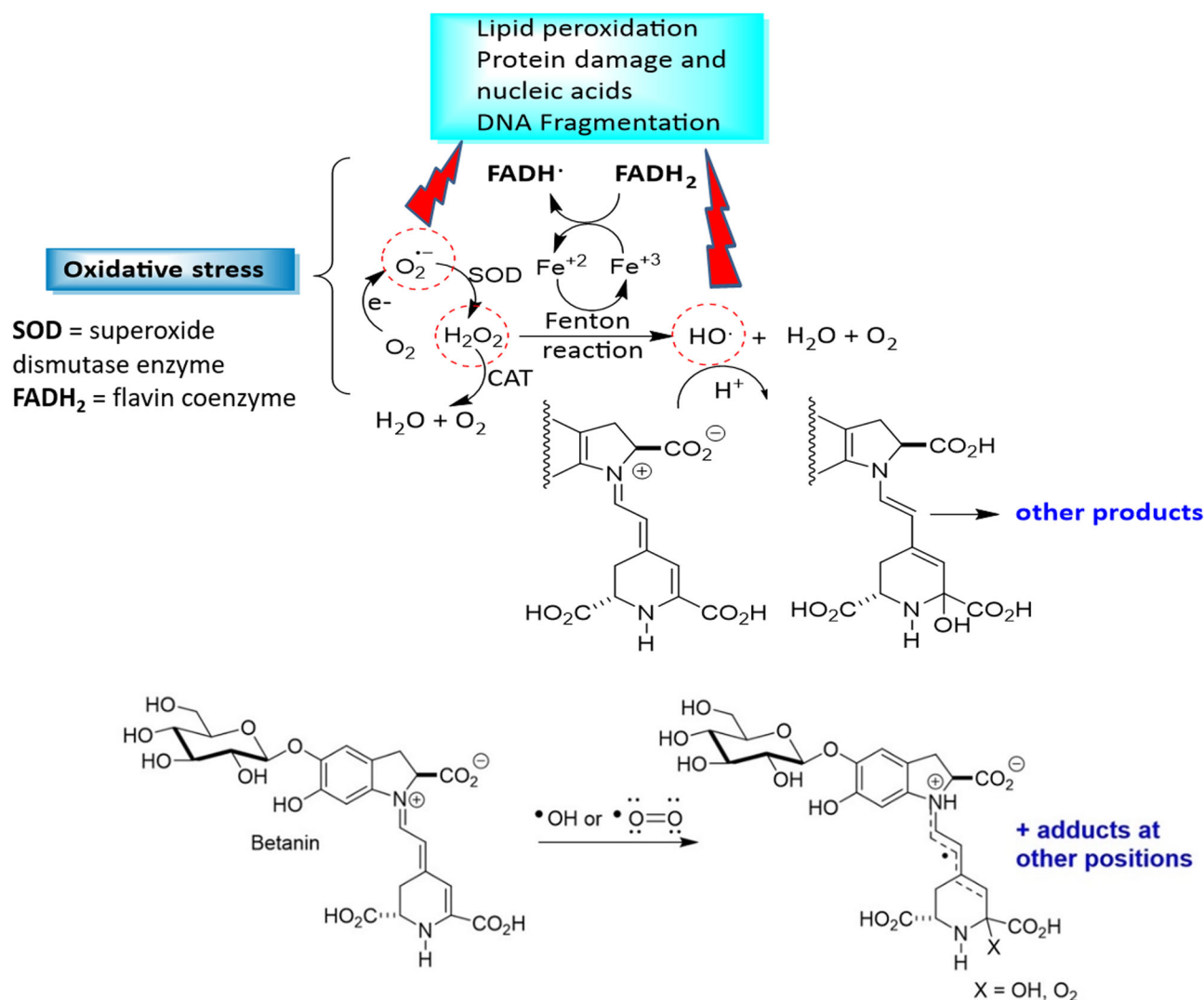


Figure 4. Betanin as a radical scavenger, hydrogen or electron donor, peroxide decomposer or singlet oxygen quencher. O₂ leakage from mitochondria generates the superoxide anion radical O₂^{•−}, which is converted to H₂O₂ by superoxide dismutase (SOD). Both, O₂^{•−} and H₂O₂ can cause damage to cellular components. H₂O₂ can be cleaved to the radical by iron-sulfur clusters found in several metalloproteins (e.g. ferredoxins, NADH dehydrogenase, hydrogenases, coenzyme Q) by increasing Fe²⁺ in the cytosol. Fe²⁺ reacts with H₂O₂ through the Fenton reaction, generating HO[•], which causes serious oxidative damage. Fe³⁺ is then reduced back to Fe²⁺ by flavoproteins (FADH₂) to generate oxidized flavin radicals (FADH[•]) and the hydroxyl radical. Betanin can also counteract with [•]OH or [•]O = O radicals forming several adducts.

disturbances of the pro-oxidant–antioxidant balance caused by abnormal ROS and RNS generation and/or impaired antioxidant defenses, establishing oxidative stress (Hackam and Anand 2003; Halliwell and Whiteman 2004).

The superoxide anion (O₂^{•−}), hydrogen peroxide (H₂O₂), hydroxyl radical (HO[•]) and peroxynitrite (O = N–O–O^{•−}) are the main oxidative molecules with potential harmful implications to human health, since at least one unpaired electron in the last electron layer is available, with this incomplete electron shell conferring high reactivity to these molecules (Burton and Jauniaux 2011; Halliwell and Whiteman 2004; Nita and Grzybowski 2016). Excessive amounts of reactive species cause oxidative damage to macromolecules such as DNA, lipids and proteins, as well as the interruption of redox-dependent signaling pathways in vasculature, leading to endothelial dysfunction and atherogenesis (Figure 4) (Evrard et al. 2016; Förstermann, Xia, and Li 2017).

Subendothelial retention of apoB100-containing lipoprotein is an early step in atherogenesis, which induces a greater consumption of NO, decreasing its bioavailability and leading to higher generation of free radicals and the establishment of an inflammatory process (Libby 2002; Lu et al. 2011; Peluso et al. 2012).

The increase in free radicals leads to the peroxidation of fatty acids in LDL particles, increasing in their negative charge, density, and lysolecithin, cholesterol oxide, hydroxide and hydroperoxide content, cytotoxicity and decreasing polyunsaturated fatty acids, with consequent fragmentation of apolipoprotein B-100 (apo B-100) (Kovanen and Pentikainen 2003). These changes in physicochemical LDL properties favor chemotactic activity for circulating monocytes and T-cells, leading to adhesion of inflammatory cells to the endothelium, the release of pro-inflammatory interleukins and cytokines and ROS, maintaining LDL oxidation (Garcia-Dorado et al. 2014). The permanent ROS influence

Table 1. Betanin effects on experimental models mimicking risk factors for CVD: Evaluation of direct and indirect antioxidant and anti-inflammatory effects.

Experimental model	Betanin dosage	Betanin effects	Study
<i>in vitro</i> (linoleate emulsion)/ <i>ex vivo</i> (human LDL)	0.4–2.5 μ M	↑ linoleate oxidation by cyt c (BET IC ₅₀ 0.4 μ M), MetHb (betanin at 1.92 μ M) and LOX-1 (betanin IC ₅₀ 0.6 μ M) ↓ LDL oxidation by MetHb (betanin IC ₅₀ < 2.5 μ M)	Kanner, Harel, and Granit 2001
<i>ex vivo</i> (human LDL)	25–100 μ M	↑ BET incorporation of 0.51 ± 0.06 nmol to LDL LDL resistance to oxidation by copper (~15 min lag phase)	Tesoriere et al. 2003
<i>ex vivo</i> (human endothelial cells)	5 μ M	↓ ICAM-1 (30 %) expression induced by TNF- α in endothelial cells	Gentile et al. 2004
<i>in vitro</i> (liposome and arachidonic acid)	100 μ g/mL	↓ Liposome oxidation (70 %) FeCl ₂ -induced ↓ COX-1 (33 %) and COX-2 (97 %) activities	Reddy, Alexander-Lindo, and Nair 2005
<i>ex vivo</i> (human LDL)	0.5–10 μ M	LDL oxidation by MPO (hydroperoxides generation is delayed by 100 min)	Allegra, Tesoriere, and Livrea 2007
<i>in vitro</i> (methyl linoleate and soybean PC model)	2–10 μ M	↓ linoleate oxidation by AMVN Dose-dependent BET ($r^2 = 0.93$) ↓ PC oxidation by AAPH dose-dependent BET ($r^2 = 0.98$)	Tesoriere et al. 2009
<i>in vitro</i> (free radical generation assay)	1–100 μ M	BET free radical scavenged OH• (40 %), O ₂ • (50 %), DPPH (25 %) and galvinoxyl (50 %)*	Esatbeyoglu et al. 2014
Animals (Sprague–Dawley rats)	25 and 100 mg·kg ⁻¹ / intra-gastric gavage	↓ TGF- β (7 and 10 %), CTGF (24 and 28 %), NADPH (20 and 27 %) and NF-kB DNA-binding (12 and 18 %) compared to high fructose treatment ↑ GPx (27 and 36 %) and GSH (38 and 45 %) compared to high fructose treatment	Han et al. 2015
Animals (Sprague–Dawley rats)	25 and 100 mg·kg ⁻¹ / subcutaneous injection	↓ Myocardial infarction size (13 and 20 %), iNOS (12 and 50 %), NF-kB (24 and 46 %) and ROS (15 and 35 %) ↑ SOD (15 and 43 %), CAT (22 and 34 %) and GSH (42 and 59 %)	Yang et al. 2016
Animals (Sprague–Dawley rats)	25, 50 and 100 mg·kg ⁻¹ / intra-gastric gavage	↓ TGF- β (26, 50 and 57 %) and MDA (14, 21, 43 %) ↑ SOD (44, 69 and 72 %) and (CAT 46, 60 and 72 %) compared to the DM group	Sutariya and Saraf 2017
Animals (Wistar rats)	20 mg·kg ⁻¹ / intra-gastric gavage	↑ GPx (37 %), CAT (40 %) and SOD (36 %) following dyslipidemia and DM induction	Silva et al. 2019b
Animals (Sprague–Dawley rats)	100 mg·kg ⁻¹ / intraperitoneal injection	↓ MDA (50 %), MPO (28 %) and IL-6 (26 %) following heart I/R injury	Tural et al. 2020a
Animals (Sprague–Dawley rats)	100 mg·kg ⁻¹ / intraperitoneal injection	↓ MDA (42 %) and MPO (23 %) ↑ GSH (30 %) after aortic clamping I/R injury	Tural et al. 2020b

BET – betanin, CAT – catalase, CD – conjugated diene, COX-1 – cyclooxygenase 1, COX-2 – cyclooxygenase 2, cyt c – cytochrome c, CTGF – connective tissue growth factor, DM – diabetes mellitus, DPPH• – 2, 2'-diphenyl- β -picrylhydrazyl radical, FeCl₂ – ferrous chloride, GPx – glutathione peroxidase enzyme, GSH – glutathione, H₂O₂ – hydrogen peroxide, ICAM-1 – intercellular adhesion molecule 1, IL-6 – interleukin-6, I/R – ischemia/reperfusion, LDL – low density lipoprotein, LOX-1 – lipoxygenase-1, MDA – malondialdehyde, MetHb – metmyoglobin, MPO – myeloperoxidase, NADPH – nicotinamide adenine dinucleotide phosphate, NF-kB – factor nuclear kappa B, OH• – hydroxyl radical, O₂• – superoxide radical, PC – phosphatidylcholine, SOD – superoxide dismutase, TGF- β – Transforming growth factor beta, TNF- α – tumor necrosis factor alpha.

*Results are expressed as a percentage relative to the control evaluated by electron spin resonance spectroscopy at the highest betanin dose.

on LDL and underlying endothelium molecules may promote an increase in vasoconstrictor substances, like thromboxane and angiotensin II, adhesion molecules and growth factors, along with the proliferation of smooth muscle cells, impairing the maintenance of homeostasis and vascular tone (Hulthe and Fagerberg 2002).

In addition, oxidized LDL may increase arginine methyltransferase I and II in endothelial cells, increasing the formation of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). ADMA inhibits the three eNOS isoforms and may also dissociate these enzymes, generating superoxides, while SDMA can compete with

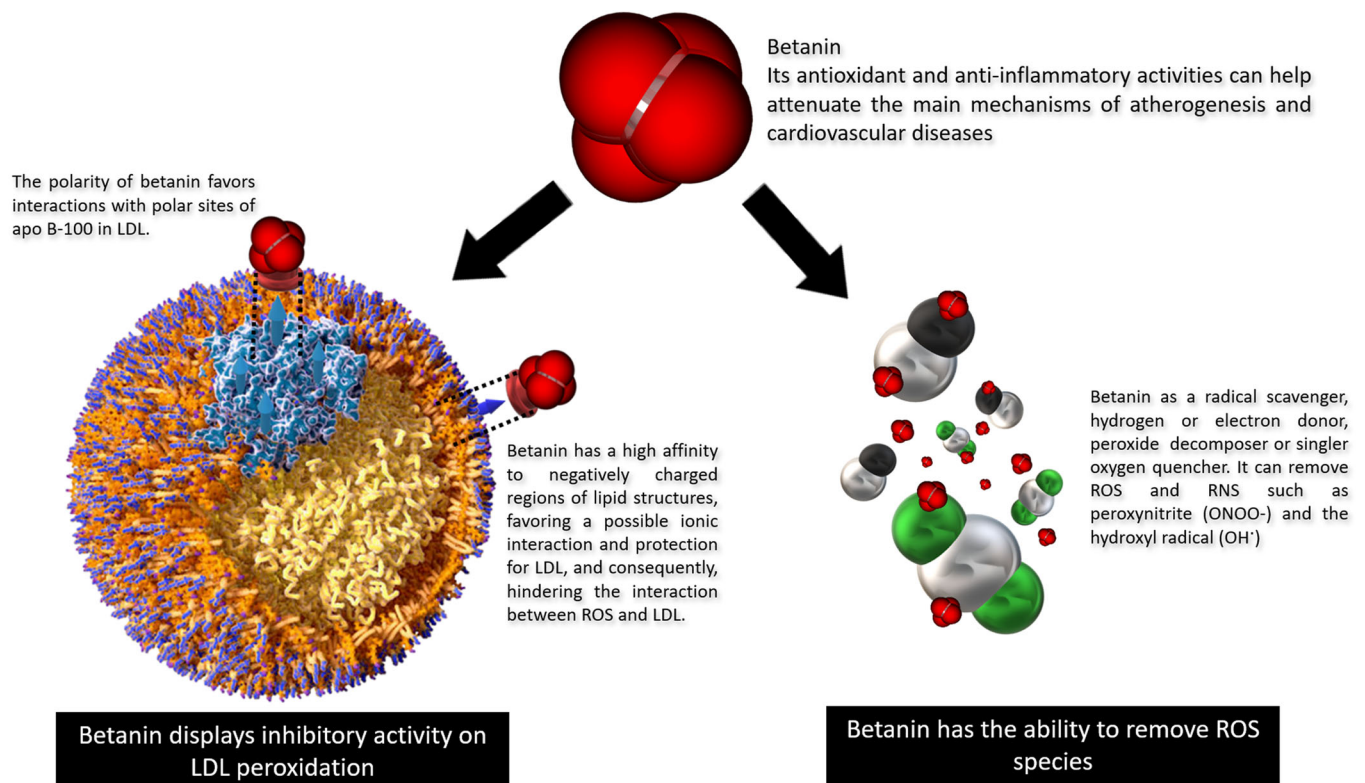


Figure 5. Betanin attenuates LDL peroxidation. Betanin antioxidant and anti-inflammatory activities can attenuate the main atherogenesis mechanisms, protecting against CVD. Betanin free-radical scavenger activity removes ROS and prevents LDL peroxidation. Betanin in the bloodstream is able to establish ionic interactions with the polar head of phospholipids and to Tyr residues in apoB-100, avoiding tyrosine generation and LDL aggregation.

arginine for the cationic amino acid transporter in the cell membrane, accentuating arginine deficiency and indirectly altering NO production levels (Smith et al. 2018).

Betanin antioxidant and anti-inflammatory activities can attenuate the main atherogenesis and cardiovascular disease mechanisms. Data included in Table 1 summarizes the studies already available, including the pre-clinical trials, which were used to embase the potential of betanin as a health-influencing compound in risk factors for CVD.

Positively charged betanin regions confer affinity to negatively charged lipid portions ($-\text{CO}_2^-$), favoring interaction with and protection of lipid membranes and LDL. In addition, this beet pigment inhibits LDL peroxidation by binding to the polar head of fatty acids or polar residues of apo B-100, protecting LDL from oxidation (Figure 5) (Kanner, Harel, and Granit 2001; Tesoriere et al. 2003; Allegra, Tesoriere, and Livrea 2007). In human LDL particles, apoB100 protein is sensitive to nitration by ROS and RNS at the tyrosine residues Tyr 413, 2524 and 3295, forming tyrosine-tyrosine bonds, also known as dityrosine crosslinks, resulting in LDL aggregates, a key event in early atherogenesis. In addition, augmented dityrosine crosslinks were detected in the plasma and LDL from human atherosclerotic lesions compared to LDL from healthy subjects (Figure 5) (Hamilton et al. 2008; Chakraborty, Cai, and Tarr 2010; Mukherjee et al. 2017; Wu et al. 2015).

In addition, betanin has the ability to interfere with the initiation of lipid peroxidation through its ability to donate electrons, first reacting with oxidizing molecules. Betanin binds to isolated LDLs from human plasma and prevents

copper-induced lipoprotein peroxidation and also protects β -carotene residents in LDL of the degradation (Tesoriere et al. 2003). In supplemented healthy volunteers with 500 g cactus pear fruit pulp, after 3 and 5 h ingestion of the fruit meal, plasma LDL was isolated and betalains were incorporated to the system. LDL particles appeared more resistant to ex vivo-induced oxidative injury, and this resistance increases with betalain concentrations (Tesoriere et al. 2004). Additionally, betanin may interact with oxidant molecules like ferric ions (Fe^{3+}), which reduce to ferrous form (Fe^{2+}) and inhibit OH^\bullet radical formation (Kanner, Harel, and Granit 2001). This radical is the main initiator of lipid peroxidation, acting through the removal of hydrogen atoms from lipid membranes, altering their permeability, resulting in loss of selectivity for the influx and efflux of nutrients and toxic substances into the cell, leading to damage and cell death (Halliwell and Whiteman 2004).

Lipid oxidation generates toxic metabolic compounds, hydroperoxides, hydroxides, isoprostanes, aldehydes and ketones, which can aggravate atherogenesis. Malondialdehyde (MDA) is formed from the peroxidation of polyunsaturated fatty acids, such as linoleic acid, and is a clinical biomarker to assess oxidative damage (Niki 2014; Ayala, Muñoz, and Argüelles 2014; Sousa, Pitt, and Spickett 2017).

Betanin effects on lipid peroxidation have been evaluated by the reduced generation of toxic metabolites in several organs and tissues, as demonstrated in preclinical trials (Silva et al. 2019b). Animals treated with betanin exhibited lower MDA in liver and kidneys after oxidative tissue

damage induced by carbon tetrachloride, paraquat, organophosphates, paracetamol or diclofenate (Sutariya and Saraf 2017; Ahmadian et al. 2018; Motawi et al. 2019).

Ischemia and reperfusion injury are common complications during CVD therapies, since the reactivation of aerobic metabolism during reperfusion induces an increase in ROS by neutrophils and the mitochondrial metabolism, resulting in damage to membranes and nucleic acids, culminating in cell death (Weigand et al. 2012; Garcia-Dorado et al. 2014; Zhou et al. 2018). Betanin prevents oxidative damage in the lung and heart of animals induced by ischemia-reperfusion injury, evidenced by decreased MDA production in tissues. The same favorable effect of betanin on MDA was observed in the abdominal aorta following ischemia and reperfusion injury (Tural et al. 2020a; 2020b). Taken together, these findings reinforce the protective effects of betanin on vascular integrity against oxidative damage.

In addition to its free-radical scavenging ability, betanin exerts its protective role on cell and vascular intima through the induction of cellular antioxidant system defenses (Silva, Pereira, et al. 2019). Risk factors for CVD are associated with reduced activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx), failing in maintaining cellular redox homeostasis (Chen et al. 2012; He et al. 2017; Sies, Berndt, and Jones 2017).

Clinical trials have demonstrated betanin's ability to modulate endogenous antioxidant systems in several oxidative stress-inducing disorders (Rahimi et al. 2019). This indicates that betanin can induce antioxidant enzymes during cardiovascular abnormalities, since redox imbalances are inherent to the physiopathology of those disorders.

In a study conducted with male Sprague-Dawley rats, five days betanin supplementation at 25 and 100 mg·kg⁻¹ dose avoided decreased SOD and CAT activities by the paraquat herbicide in hepatic and renal tissues (Han et al. 2014; Tan et al. 2015).

In an experimental model for diabetes mellitus, female Sprague-Dawley rats supplemented with betanin at 50 and 100 mg·kg⁻¹ for 8 weeks restored renal SOD and CAT activities (Sutariya and Saraf 2017).

Betanin supplementation antagonized the anti-fibrotic effects of diabetic cardiomyopathy induced by high fructose intake in a dose-dependent manner (25 and 100 mg·kg⁻¹). The cardiac cells of male treated Sprague-Dawley rats exhibited decreased protein glycation accompanied by increased GPx activity (Han et al. 2015).

Betanin offered for 20 days at 20 mg·kg⁻¹ significantly restored the redox status in Wistar rats by up-regulating SOD, CAT and GPx enzymatic activities evaluated in the hepatocytes of animals with hypercholesterolemia, hyperglycemia and hepatic steatosis induced by a hypercaloric and hyperlipidic diet (Silva, Pereira, et al. 2019). Similarly, plasmatic SOD and CAT activities were restored by betanin intake in an experimental rat acute myocardial infarction model (Yang et al. 2016).

Oxidative stress and inflammation are key and closely related processes in the pathogenesis of atherosclerosis and

CVD, since the high generation of ROS causes vascular damage and tissue inflammation, leading to endothelial dysfunction, favoring atherogenesis (Siti, Kamisah, and Kamsiah 2015; Guzik and Touyz 2017). In addition to the ability to remove ROS and modulate antioxidant enzymes, betanin has been shown to play an important role in anti-inflammatory responses (Vidal et al. 2014).

Betanin was shown to inhibit the inflammatory enzymes cyclooxygenase 2 (COX-2), and cyclooxygenase 1 (COX-1) in vitro by 97% and 33.5%, respectively (Reddy, Alexander-Lindo, and Nair 2005). Betanin was more effective in inhibiting COX-2 than other phytochemicals, such as cyanidin-3-O-glycoside (anthocyanin), lycopene, chlorophyll, β -carotene and bixin. Since COX-2 is induced in injury and inflammation conditions, while COX-1 is constitutively expressed under physiological conditions in various tissues, catalyzing the cytoprotective prostaglandin biosynthesis (PGI₂ and PGE₂), mainly in the gastrointestinal system, the apparent selectivity of betanin over COX-2 resembles the COX-2 selective non-steroidal anti-inflammatory drugs (NSAIDs), which avoid the deleterious effects of unwanted COX-1 inhibition (gastrointestinal, renal and liver toxicity) (Ferrer et al. 2019; Radi and Khan 2019; Süleyman, Demircan, and Karagöz 2007; Tai and McAlindon 2018; Cooper et al. 2019).

Lipoxygenases (LOX) seems to be another target for betanin-derivative compounds. The enzymes involved in the biosynthesis of leukotrienes, that act in chemotaxis and leukocyte adhesion to endothelial cells and neutrophil degranulation, among other events are inhibited by betanidin (betanin aglycone), but not betanin, which is a potent LOX-1 inactivator by binding to specific amino acid residues on the surface of LOX-1, near the active site, interfering in enzyme-substrate interaction (Rådmark et al. 2015; Vidal et al. 2014). Therefore, although betanin is not as effective in inhibiting LOX-1, it is the precursor for betanidin, generated by the hydrolysis of the glycosidic bond at a systemic level. Further studies regarding this mechanism are required.

In in vitro model of endothelial cells obtained from the human umbilical vein, betanin (5 μ M) was shown to protect endothelial cells from a cytokine-induced redox imbalance, by inhibiting the expression of the proinflammatory intercellular adhesion molecule 1 (ICAM-1), which causes leukocyte recruitment, pro-inflammatory gene expression and endothelial dysfunction (Gentile et al. 2004; Engin 2017; Marcos-Ramiro, García-Weber, and Millán 2014).

In male Swiss mice, betanin (100 mg·kg⁻¹) administered through subcutaneous and oral routes inhibited the formation of paw edema and recruitment and levels of leukocytes, mononuclear cells and neutrophils after an inflammation stimulus. Betanin also reduced the production of the inflammatory cytokines, TNF- α and IL- β , and increased levels of IL-10 (Martinez et al. 2015). As TNF- α and IL- β are crucially involved in multiple inflammatory cardiac pathologies, such as heart failure, myocardial infarction and cardiomyopathies, they have been considered targets for treatments against cardiovascular events. These cytokines induce adhesion molecule expressions in the endothelium, like the

aforementioned ICAM-1, that promote leukocyte leakage and can cause systemic inflammatory response and pathological abnormalities (Kleinbongard, Heusch, and Schulz 2010; Libby 2017). However, clinical trials have confirmed the anti-inflammatory and cardioprotective role played by IL-10, that should be included as a therapeutic target, due to decreases in TNF- α and IL- β secretion, among other cytokines, as well as improved cardiac function, reduced myocardial infarction and ischemia and reperfusion injury (Bhattacharyya et al. 2013; Zhang, Xing, et al. 2015; Chang et al. 2015; Bartekova et al. 2018). Betanin displays a similar effect to IL-10-inducing drugs and, consequently, antagonizes the secretion of pro-inflammatory cytokines (Zhou et al. 2005).

Betanin also reduces the expression of inducible nitric oxide synthase (iNOS) and confirmed the inhibitory effect on cyclooxygenase 2 (COX-2) in the kidneys of Dawley rats in an acute oxidative stress and inflammation experimental model (Tan et al. 2015).

Although several evidences indicate that betanin alleviates the inflammatory response, the molecular mechanisms by which it exerts its pharmacological effects still require elucidation. It can be hypothesized that betanin effects are mediated through the nuclear factor transcription factor kappa beta (NF- κ B), a common modulator of several genes encoding pro-inflammatory chemokines and cytokines. Betanin influence on the NF- κ B pathway will be discussed further ahead.

Finally, betanin has been shown to play a specific role in insulin resistance and type 2 diabetes mellitus, both posing a significant risk for the development of atherosclerotic diseases, including coronary heart disease, peripheral arterial disease, and cerebrovascular disease (Aronson and Edelman 2014; Thiruvoipati, Kielhorn, and Armstrong 2015; Ergul et al. 2012). Betanin administered at 25 and 100 mg·kg⁻¹ for 60 days was able to revert hyperglycemia, hyperinsulinemia, HOMA-IR and glycation products in animals exhibiting experimental diabetes induced by high fructose ingestion (Han et al. 2015). Betanin at a concentration of 20 mg·kg⁻¹ administered to rats was shown to alleviate the effects of experimental type 2 diabetes induced by the intraperitoneal administration of STZ and NA. Betanin exerted its effects by regulating liver glucose metabolism-related enzymes, such as glucokinase, glucose-6-phosphatase, pyruvate kinase, glucose-6-phosphate dehydrogenase, and fructose-1,6-bisphosphatase (Dhananjayan et al. 2017).

Corroborating these findings, after short-term administration (20 days), betanin modulated the glucose metabolism, insulin production and reversed insulin resistance induced in rats fed by a high fat hypercaloric diet (Silva, Pereira, et al. 2019).

Betanin as a putative activator of the anti-oxidative Nrf2-ARE pathway and supressor of the anti-inflammatory NfK-B pathway in CVD

As mentioned previously, oxidative stress, inflammation and endothelial dysfunction are common conditions in the

pathophysiology of several cardiovascular outcomes, such as atherosclerosis (Quirós-Fernández et al. 2019; Förstermann, Xia and Li, 2019), heart failure (Ramirez-Sanchez et al. 2013; Koning et al. 2016; Lubrano and Balzan 2020), myocardial hypertrophy (Wu et al. 2012; Wilder et al. 2015) and ischemia and reperfusion injury (Tian et al. 2015; Granger and Kvietys 2015).

Some health-promoting compounds, like betanin, can interfere in the activation of transcriptional factors, i.e. the nuclear factor kappa B (NF- κ B) and the factor 2 related to nuclear erythroid factor 2 (Nrf2), master regulators in homeostasis redox, which coordinate the RNA synthesis of numerous genes involved in inflammatory, cytoprotective and antioxidant responses (Siti, Kamisah, and Kamsiah 2015; Lingappan 2018; Liu et al. 2019; Baird and Yamamoto 2020; Sireesh et al. 2018; Sies, Berndt, and Jones 2017).

NF- κ B is the major gene expression regulator in inflammatory processes. Under normal physiological conditions, NF- κ B is inactive in the cytoplasm and linked to inhibitory proteins, known as κ B inhibitors (I κ B α), (Hayden and Ghosh 2012; Giuliani, Bucci, and Napolitano 2018). On the other hand, under cellular stress conditions such as UV radiation and mainly oxidative stress (H₂O₂), kinases called IKK are activated and result in the phosphorylation and degradation of I κ B α (Allen and Tresini 2000; Yin et al. 2015; Zambrano et al. 2016). The dissociation of the I κ B α /NF- κ B complex culminates with the translocation of NF- κ B to the nucleus. In the nucleus, NF- κ B binds to the promoter region of target genes, initiating the transcription and expression of a range of inflammatory cytokines, chemokines and adhesion molecules involved in the development and progression of inflammatory responses and increased cardiovascular risks (Zambrano et al. 2016; Liu et al. 2017; Fiordelisi et al. 2019).

Cells respond to the deleterious effects of pro-oxidative and pro-inflammatory environments by activating Nrf2, a transcriptional factor modulator of genes involved in cellular redox homeostasis (Cuadrado et al. 2018). Nrf2 is inactive in the cytoplasm and bound through the DLG and ETGE sequences in the Neh 2 domain to the N-terminal region of the Kelch-like repressor protein ECH-associated protein 1 (Keap1) (Tonelli, Chio, and Tuveson 2018). Keap1 is rich in Cys residues in the N-terminal region, in its BTB (bric-à-brac) and IVR (intervening region) domains, as well as in the C-terminal region, which are sensitive to electrophiles and oxidants (Kansanen et al. 2013; Dinkova-Kostova, Kostov, and Canning 2017). When in oxidative stress, its Cys residues, mainly Cys151, 273 and 288, are oxidized, resulting in Keap1 inactivation and Nrf2 derepression (Buendia et al. 2016; Saito et al. 2016; Gao et al. 2020). Nrf2 then translocates to the nucleus, where it binds specifically to ARE (antioxidant response element) sequences in the promoter region of several genes, encoding enzymes involved in cytoprotective and antioxidant effects (Hayes and Dinkova-Kostova 2014; Tonelli, Chio, and Tuveson 2018).

Nrf2 is a potential target for therapeutic purposes to treat vascular disorders induced by oxidative stress, through the

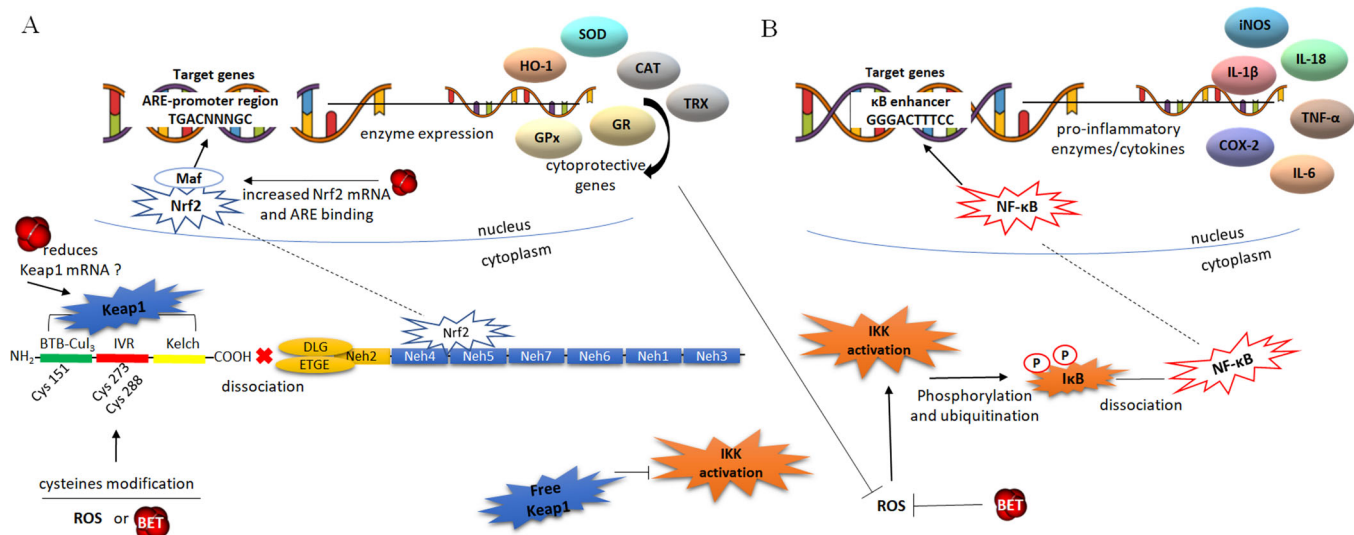


Figure 6. Putative effects of betanin on the redox sensitive Nrf2-ARE and NF- κ B pathways. Panel A -The transcription factor Nrf2 is bound to Keap1 in the cytoplasm, and the Nrf2-Keap1 complex dissociation allows for Nrf2 translocation to the nucleus. In the nucleus, Nrf2 dimerizes with the Maf protein and binds to ARE sequences in the promoter of genes that encode phase II detoxification and antioxidants enzymes. Betanin can down-regulate Keap1 expression and/or modify Cys residues culminating in the dissociation of Nrf2, which, in turn, translocates to the nucleus. Betanin can also cross-link the two pathways by up regulating the expression of Nrf2 in the nucleus, favoring the transcription of antioxidant/cytoprotective genes. Panel B -The NF- κ B transcriptional factor is synthesized and constitutively linked to the inhibitory protein I κ B α in the cytoplasm. The dissociation of the I κ B-NF- κ B complex occurs by phosphorylation of I κ B by IKK kinases which are then activated in response to ROS and oxidative stress. I κ B phosphorylation results in its ubiquitination and dissociation of NF- κ B, which translocates to nucleus. The activated NF- κ B binds to the promoter of target genes, activating the transcription of inflammatory cytokines, chemokines and adhesion molecules. Betanin acts directly on ROS and on antioxidant genes, subsequently inhibiting the activation of IKK, avoiding I κ B phosphorylation and release of NF- κ B to the nucleus. Thus, betanin, through its role on the dissociation of Keap1-Nrf2, makes Keap1 free for inhibitory binding with IKK. Legend: ARE – Antioxidant response element, BET betanin, BTB bric-à-brac protein, CAT – catalase, COX-2 – cyclooxygenase 2, Cys – cysteine residues, GPx – glutathione peroxidase, GR – glutathione reductase, HO-1 – heme oxygenase 1, IKK – i κ B kinase, iNOS – inducible nitric oxide synthase, IL – interleukins, IVR – intervening region, Keap1 – Kelch-like ECH-associated protein 1, Maf – small musculoaponeurotic fibrosarcoma protein, Nrf2 – nuclear factor erythroid 2-related factor 2, NF- κ B – kappa B nuclear factor, P – phosphorylation, SOD – superoxide dismutase, TRX – thioredoxin, TNF- α – tumoral necrosis factor alpha.

overexpression of Nrf2 or its dissociation from the Keap1-Nrf2 complex, protecting against CVD (Cuadrado et al. 2019; Chen and Maltagliati 2018; Ge et al. 2019). Food antioxidants have been considered as adjuvants for the treatment of cardiovascular diseases due to the activation exerted on Nrf2 and antioxidant response elements (ARE) (Ooi et al. 2018; Matzinger, Fischhuber, and Heiss 2018; Li and Zhang 2017).

Betanin health-promoting benefits to the cardiovascular system are due to its anti-radical scavenger effect that stabilizes the reactivity of these molecules, protecting from endothelial tissue damage, but simultaneously down-regulating the mRNA of pro-inflammatory mediators while reinforcing endogenous antioxidant defenses. Such properties contribute to reduce endothelial cell ROS-mediated damage and inflammation to.

Betanin influences gene expression through the Nrf2-ARE and NF- κ B signal transduction pathways, pointed out as the mechanisms underlying its indirect effects. Betanin has been reported as a NF- κ B DNA-binding suppressor, followed by inflammatory cytokines COX-2, iNOS and IL-1 β gene transcription down-regulator (Tan et al. 2015; Han et al. 2015). By acting in a pro-oxidative environment, i.e. containing high H₂O₂ concentrations, betanin can avoid translocation of the NF- κ B to the nucleus by inhibiting i κ B/NF- κ B complex dissociation (Esatbeyoglu et al. 2014; Zielińska-Przyjemaska et al. 2012; Oliveira-Marques et al. 2009) (Figure 6). Corroborating these findings, betanin was shown to reduce ROS and downregulate NF- κ B expression

in acute myocardial infarction conditions induced by isoproterenol (Yang et al. 2016).

Liver cells treated by betanin exhibit a slight reduction in Keap 1 mRNA in the cytosol and increased nuclear Nrf2 expression and levels. In addition, betanin favors the binding of Nrf2 to the ARE sequence, and up-regulation of Nrf2 target genes encoding cellular phase II detoxification and antioxidant/cytoprotective enzymes, such as glutathione S-transferases GSTP, GSTM, GSTT, GSTA and NQO1 (NAD(P)H quinone dehydrogenase 1) (Krajka-Kuzniak et al. 2013).

Betanin induces Nrf2 transactivation accompanied by an increase in heme oxygenase 1 (HO-1), paraoxonase 1 (PON1) and glutathione (GSH,) in a dose-dependent manner (Esatbeyoglu et al. 2014). PON1 exhibits antioxidant, anti-inflammatory and anti-atherogenic properties, circulating in the plasma bound to HDL and, at the same time, delays and/or prevents LDL oxidation, thus mediating anti-atherogenic effects. In addition, low PON1 levels in human plasma are associated with increased risks of heart disease (Schrader and Rimbach 2011; Wang et al. 2012; Litvinov, Mahini, and Garelnabi 2012). GSH, the most abundant antioxidant in the heart, protects against oxidative stress, myocardial infarction and coronary artery disease at physiological levels (Bajic et al. 2019).

These reports evidence how betanin supports the antioxidant defense system and how it impacts CVD.

The Keap1 protein may be the main betanin target concerning activation of the Nrf2-ARE pathway. Similar to that

described for other bioactive compounds, betanin is able to modify cysteine residues, specifically Cys 151, 273 and 288, through interaction with their sulfhydryl groups (SH), thus allowing for the breakdown of the Keap1-Nrf2 interaction, a putative mechanism like the one already described for sulphoraphane found in broccoli (Hu et al. 2011). In addition, as reported in a recent review, several antioxidant compounds, such as the flavonoid rutin, can modify Keap1 cysteine residues, through oxidation and alkylation, thereby activating Nrf2/ARE pathway (Ooi et al. 2018).

As pointed out, betanin seems to also be involved in mitogen-activated protein kinase (MAPK) activation which, in turn, phosphorylates Nrf2, increasing its stability, and promoting nuclear translocation and transactivation (Krajka-Kuzniak et al., 2013).

Another way through which betanin may alleviate the oxidative stress-inflammation-CVD triad is by inhibition of the NF- κ B pathway through a positive effect on Nrf2-ARE, joining the two pathways in a coordinated manner. In the NF- κ B pathway, the protein kinase IKK binds to the ETGE sequence (one of the sequences by which Nrf2 is constitutively linked to Keap1), so that IKK can bind to free Keap1, making it impossible to phosphorylate I κ B and dissociate the I κ B-NF- κ B complex. Consequently, NF- κ B translocation to the nucleus and inflammatory gene transcription does not occur (Kim et al. 2010; Stefanson and Bakovic 2014). If betanin increases the Nrf2 translocation to the nucleus (Krajka-Kuzniak et al. 2013), the intracellular levels of free Keap1 (dissociated from Nrf2) would become higher and available for interaction with IKK, thus inhibiting NF- κ B activation (Figure 6).

In sum, betanin can modulate oxidative stress and reduce inflammation through multiples pathways, preventing the major events for the progression of CVD. The putative molecular mechanisms of betanin, not all proven so far, include (Figure 6): (i) ROS and RNS scavenging by electron and H⁺ donation; (ii) preventing both peroxidation of phospholipids and apoB-100 in LDL particles, and may also prevent the formation of dityrosine, avoiding the aggregation of LDL particles, which lead to endothelial dysfunction and trigger atherogenesis; (iii) inhibition of NF- κ B pathway activation by directly remove ROS; (iv) down regulation of Keap1 expression and/or betanin interaction through Keap1 cysteine residues, favoring the translocation of Nrf-2 to the nucleus; (v) putative enhancement of the Keap1-Nrf2 complex dissociation, making Keap1 available for IKK binding and then inhibiting NF- κ B activation; (vi) up-regulation of Nrf2, activating the endogenous antioxidant response which, in turn, attenuates oxidative stress and prevents NF- κ B activation.

Conclusions

Betanin seems to be a supportive therapeutic alternative to attenuate the main mechanisms involved in CVD by oxidative stress and inflammation, without any harmful effects. Betanin contributes to restore the oxidative homeostasis and mitigate vascular inflammation. Betanin bioavailability

reaching plasma in physiological concentrations with retention of the original structure is guaranteed by the physico-chemical characteristics of the bloodstream. Although the exact mechanisms by which betanin exerts its cardioprotective role have not been yet fully elucidated, its ability to act directly on ROS/RNS species alongside the induction of the antioxidant/cytoprotective Nrf2-ARE pathway and suppression of the inflammatory NF- κ B pathway in CVD can account for all of betanin health-promoting benefits.

However, the molecular mechanisms of betanin modulation regarding gene expressions and cell signaling pathways is a new and challenging issue and, thus, is speculative due to the limited data available in the literature. For this reason, further *in vitro* and even *in silico* tests can contribute to elucidate the exact manner in which betanin activates the Nrf2-ARE pathway and suppresses the NF- κ B pathway. In addition, since betanin is bioaccessible, bioavailable, approved for use in foods in *quantum satis*, and has not shown any harmful or deleterious effects in animals, clinical trials should be conducted to determine the effective dose and supplementation regimen to achieve the desired therapeutic outcomes in human beings.

Author contributions

DVT, DSB and VMFP conceptualized and designed the manuscript, participating in drafting the article and/or acquisition of data, and/or analysis and interpretation of data; DVT, DSB and VFF prepared the figures and tables. DVT, DSB, VFF and VMFP wrote, edited and revised the manuscript critically. VMFP revised the final written. All authors critically revised the manuscript concerning intellectual content and approved the final manuscript.

Disclosure statement

The authors declare no conflicts of interest.

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