# Phytochemical activators of Nrf2: a review of therapeutic strategy in diabetes

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Review

Phytochemical activators of Nrf2: a review of therapeutic strategies in diabetes

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Abstract

Insulin resistance (IR) is fundamental to the development of type 2 diabetes (T2D), and altered mitochondrial function and abnormal lipid distribution are closely associated with IR or T2D. Excess oxidative stress-induced mitochondrial damage leads to an imbalance in redox homeostasis, which is considered the major contributor to the progression of diabetes. A key cellular defense mechanism, namely, the nuclear factor-E2 p45-related factor 2 (Nrf2)-antioxidant response element (ARE) pathway, plays an essential protective role in combating excess oxidative stress. A series of phytochemicals are reported to improve IR and restore mitochondrial function against excess oxidative stress by activating the Nrf2-ARE signaling pathway to maintain cellular reactive oxygen species (ROS) homeostasis. The present review focuses on key knowledge gaps in the Nrf2-ARE system targeted by phytochemicals and its correlation to diabetes both in the *in vitro* and *in vivo* models and recent achievements in human clinical trials to evaluate its efficiency and safety. In addition, we provide an overview of recent research progress in nutrigenomics, precision nutrition and the interactions occurring in gut microbiota associated with the Nrf2-ARE signaling pathway and diabetes chemoprevention by phytochemicals and finally propose a future research strategy for regulating redox and microbiota balance via the Nrf2-ARE pathway. The present review aims to help us comprehensively understand the critical chemopreventive role of the Nrf2-ARE pathway targeted by phytochemicals in diabetes.

**Keywords:** phytochemical, Nrf2-ARE, insulin resistance, diabetes, chemoprevention, gut microbiota
1 Introduction

Phytochemicals are defined as bioactive nonnutrient plant secondary metabolites in fruits, vegetables, grains and other plant foods. These compounds have discrete functionalities towards animal biochemistry and metabolism and are being widely investigated for their roles in preventing and treating chronic disorders, such as diabetes mellitus [1]. An increase of diabetic patients worldwide has been estimated from 451 million to 693 million from 2017 to 2045, which led to an approximately 850 billion US dollar healthcare expenditure in 2017, making it one of the most significant contributors to healthcare costs [2]. Type 2 diabetes mellitus (T2D) accounts for more than 90% of diabetic patients and is characterized by deficient insulin secretion by pancreatic islet $\beta$-cells under impaired insulin sensitivity, namely, insulin resistance (IR) [3]. Increased IR leads to progressive glucose homeostasis dysfunction [4]. Moreover, the prevalence of diabetes will inevitably result in the intensification of diabetic-related complications such as neuropathy, retinopathy and cardiovascular diseases. The causes of diabetes remain unclear, as it is generally believed that the risk is highly associated with genetic and environmental factors [5].

Previous studies have shown that IR and diabetes are effectively treated by various phytochemicals, which regulate the nuclear factor-E2 p45-related factor 2 (Nrf2) signaling pathway to play a crucial role in the maintenance of redox and metabolic homeostasis by modulating cellular antioxidants and inflammatory stress [6]. The multiple mechanisms of the regulation of the Nrf2 system by dietary phytochemicals, especially polyphenols [quercetin, epigallocatechin 3-gallate (EGCG), baicalein, resveratrol, etc.], isothiocyanates (sulforaphane, 6-(methylsulfinyl)hexyl, isothiocyanate, etc.), organosulfur compounds (diallyl disulfide (DADS), diallyl trisulfide (DATS), etc.) and miscellaneous, were previously reviewed [7]. Recently, both in vitro and in vivo models, including cell, animal, and even human models, were used to evaluate the capacity of dietary phytochemicals to regulate...
diabetes [8,9]. However, there is a lack of a comprehensive review of phytochemicals on diabetes through β-cell dysfunction, IR and mitochondrial function for the consideration of the Nrf2 signaling pathway. Therefore, this review is focused on the molecular targets of naturally bioactive compounds in edible plants to discuss their protective/therapeutic roles in the antioxidative and anti-inflammatory regulation of diabetes by targeting the Nrf2 signal transduction pathway, which contributes to the utilization of dietary phytochemicals for the intervention of diabetes and the maintenance of human health.

2 Redox Homeostasis in T2D and Physiologic Regulation of the Nrf2-ARE Pathway

Oxidative stress refers to an imbalance between oxidative and antioxidative systems as a result of the excessive level of reactive oxygen species (ROS). The overproduction of ROS is often associated with structural and functional changes in cellular proteins and lipids, leading to cellular dysfunction, including impaired energy metabolism and cellular signalling and transport. T2D is a metabolic disorder associated with hyperglycemia and IR in which oxidative stress is generally considered as a primary cause [10]. Excessive glucose metabolism induces overproduction of ROS, which stimulates the pathways responsible for hyperglycemic and mitochondrial oxidative damage [11]. Excessive oxidative stress and prolonged exposure to ROS are the underlying factors associated with decreasing glucose uptake and glycogen synthesis by affecting the expressions of insulin signalling and glucose metabolism transporters [12]. For example, mitochondrial ROSs attenuate insulin action and abolish insulin-simulated glucose transporter 4 (GLUT4) translocation [12]. Insulin action fosters glucose uptake and suppresses lipolysis, and insulin signaling promotes glycogen synthesis through AKT in the liver [12]. Intracellular lipid moieties such as sn-1,2-diacylglycerol (DAG) that are accumulated in the plasma membrane are responsible for triggering defects in insulin action in the liver, muscle and adipocytes [12,13]. There are
several pathways that are linked to this oxidative damage, including the activation of protein kinase C (PKC) isoforms via de novo synthesis of the lipid second messenger DAG, increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation, increased polyol pathway flux, and increased production of angiotensin [14]. Elevated free fatty acids (FFAs) suppress both hepatic and peripheral insulin action because the loss of effectiveness of glucose resulted from endogenous glucose production inhibition and glucose uptake enhancement contributes importantly to fasting hyperglycemia in T2D [15]. ROS provoked by hyperglycemia and free fatty acids leads to the activation of several signaling pathways, including NF-κB, p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK), which cause chronic inflammation and the production of a series of cytokines by suppressing the secretion of insulin and promoting cell dysfunction [16] and eventually lead to T2D. Oxidative stress is a significant contributor to the development of issues in IR and T2D [17]. As shown in Figure 1, excessive oxidative stress and prolonged exposure to ROS are the underlying factors associated with mitochondrial dysfunction and hyperglycemia, resulting in diabetes. The activation of Nrf2 signaling pathway by various phytochemicals enhanced the amount of phase II enzymes, inhibited the level of ROS and protected mitochondrial and β cell functions. Thus, understanding the pathogenesis of T2D and controlling ROS levels are crucial for the prevention of T2D.

Nrf2, which responds to increased ROS levels, binds to its central negative modulator kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm and plays an essential role in the induction of genes encoding antioxidant and detoxification enzymes and a central role in the day-to-day biological response to oxidative stress [18] (Figure 2). Under homeostatic conditions, Nrf2 is kept at an off position bound to endogenous Keap1, which functions as an E3 ubiquitin ligase and constantly targets Nrf2 with Cul3-Rbx1 for ubiquitination and proteasomal degradation. In the case of oxidative stress, Nrf2 detaches from Keap1 and
translocates to the nucleus, where it heterodimerizes with Maf, and heterodimers recognize and bind to antioxidant response elements (AREs), affecting the expression of a variety of genes, such as antioxidant enzymes, including heme oxygenase 1 (HMOX-1), y-glutamylcysteine synthetase (y-GCS), peroxiredoxin (PRDX) 1, glutathione reductase (GR), thioredoxin reductase (TXNRD1) or sulfiredoxin (SRXN), drug metabolizing and detoxification enzymes [NAD(P)H quinone dehydrogenase 1 (NQO1), glutathione-S-transferase (GST), UDP-glucuronosyltransferase (UGT)] or metabolic enzymes and regulators [glucose-6-phosphate dehydrogenase (G6PDH), transketolase, malic enzyme, RXRα, PPARγ-coactivator 1 β (PGC1-β)] [19,20]. The Nrf2 activator dimethyl fumarate is reported to significantly accelerate impaired diabetic wound healing by ameliorating diabetes-mediated oxidative stress and inflammation in rat macrophage cells incubated with 25 mM glucose [21]. Thus, the activation of Nrf2 may provide a new strategy for preventing and treating diabetes by the utilization of natural activators.

3 Phytochemicals Improve IR by Activating the Nrf2-ARE Pathway in Cell Models

IR is the pathophysiologic factor of T2D [22]. High glucose, glucosamine, palmitic acid, H₂O₂, oleic acid, and streptozocin were used to induce IR in different cell models, including HepG2 cells, L02 cells, C2C12 cells, INS-1 cells and 3T3-L1 adipocytes. As the cell model is relatively easy to establish, a series of phytochemical compounds were used for ameliorating IR and oxidative stress, including ellagic acid, EGCG, benzyl isothiocyanate (BITC), phenethyl isothiocyanate, curcumin, dihydrocurcumin, silibinin, C-glycosides, daphnetin, tartary buckwheat flavonoids, naringenin, vitexin, morin and aspalathin [23–25]. ROSs are significantly downregulated by treatment with these bioactive compounds to reduce oxidative stress.

Phenethyl isothiocyanate enhanced the protein expression of Nrf2 by nearly 3-fold
compared with H$_2$O$_2$-induced 3T3-L1 cells, whereas dihydrocurcumin increased its expression by approximately 2.5 folds and 1.8 folds in HepG2 and L02 cells, respectively [23,24]. It was found that a promising new target of miR-233, which was elevated by ellagic acid (15 and 30 μM), may regulate the insulin resistance substrate 1 (IRS-1)/AKT/extracellular regulated protein kinase (ERK) pathway and oxidative stress through the Keap1/Nrf2/HO-1 system to affect the pathological process of T2D [26]. Furthermore, miR-223 mimic and inhibitor transfection were used to confirm that ellagic acid activated the Keap1-Nrf2 system by elevating miR-223 [26]. The phytochemical EGCG stimulated the nuclear translocation of Nrf2 by provoking the IRS-1/P13K/AKT/GLUT4 signaling pathway and promoted the expression of the antioxidant enzymes SOD, SOD2 and GTP [27,28]. Curcumin upregulated the protein expression of the Nrf2 system by repressing inflammatory signaling-mediated Keap1 expression under IR conditions [29].

BITC maintained glucose homeostasis by inhibiting glucose uptake and phosphorylation of IRS-1, AKT, and TBC1D1 in response to insulin and upregulated the expression of HO-1, GSTP, and glutamate cysteine ligase modifier subunit (GCLM) at the mRNA and protein levels as well as GSH content, which attenuated oxidative damage [30]. Knockdown of Nrf2 abrogated BITC enhancement of antioxidant defense and subsequently reversed its protection against palmitic acid-induced IR [30]. Moreover, BITC upregulated the gene and protein expressions of GLUT4, PPARγ, and C/EBPα [30]. In MIN6 cells, naringenin activated the protein expression of Nrf2 and its target genes GST and NQO1, thereby inhibiting cellular apoptosis [31]. Modulations by vitexin, morin and aspalathin were determined for Nrf2 protein expression and its antioxidant enzyme activities in INS-1 cells [32–34].

Glucose and lipid metabolism disorders are highly associated with T2D. In an oleic acid-induced HepG2 cell model, silibinin activated the CFLAR-JNK pathway and thereby regulated its downstream target genes involved in lipid metabolism, glucose uptake
[phosphatidylinositol 3-kinase (PI3K)-AKT] and oxidative stress [35]. Silibinin and daphnetin both decreased the protein expression of sterol-regulatory element binding protein 1C (SREBP-1C), patatin-like phospholipase domain containing 3 (PNPLA3), cytochrome P450 family 2 subfamily E member 1 (CYP2E1) and cytochrome P450 family 4 subfamily A (CYP4A), whereas PI3K-AKT and Nrf2 were increased [35, 36]. A potent effect in Nrf2 activation by daphnetin was associated with the phosphorylation of adenosine 5’-monophosphate-activated protein kinase (AMPK) [36]. Vitexin restored pancreatic β-cell function and improved the insulin signaling pathway by regulating IRS-1, IRS-2 and GLUT2 at the protein level [32]. The enhancement of insulin secretion may be attributed to the activation of the key proteins NF-κB and Nrf2 for the regulation of apoptosis in β-cells [32]. However, C-glycosides from *Apios americana* leaves reduced the protein expressions of Nrf2, HO-1 and NQO1, possibly because the threat of oxidative damage has already been posed by high glucose [37]. Hyperglycemia-induced ROSs accumulation cause sustained MAPK (JNK and p38) activation, and the underlying mechanism of the relationship between MAPKs and Nrf2 still needs further investigation [37].

4 Phytochemicals Ameliorate Glycometabolic Disorders by Activating the Nrf2-ARE Pathway in Animal Models

The development of T2D is accompanied by a chronic increase in oxidative stress in pancreatic β cells, causing cell damage and β cells mass reduction. The Nrf2 signaling pathway is considered as the captain of β cells fate in maintaining β cells redox balance, increasing β cells survival, preserving β cells function and promoting β cells proliferation [38]. Insulin is a peptide hormone produced by the β cells of the pancreatic islets of Langerhans that regulates the metabolism of carbohydrates, lipids and proteins to maintain normal blood glucose levels [39]. Insulin acts by binding to its receptor (insulin receptor) on
the membrane in skeletal muscle and adipose tissues to induce glucose uptake, whereas it decreases the glucose output in hepatic tissues. Insulin stimulates autophosphorylation of the insulin receptor, and then tyrosine phosphorylates the insulin receptor substrate, where insulin receptor proteins activate the PI3K/AKT pathway. The close association of oxidative stress and inflammation with IR and the onset of diabetes has become increasingly evident. Nrf2 is activated in response to oxidative stress and inflammation and is emerging as a critical target for combating IR, and its role in IR was recently reviewed [40]. A number of phytochemicals have shown effects on β cells improvement, IR prevention and enzyme modulation through Nrf2 signaling (Table 2).

4.1 Effect of EGCG in the diabetic animal model

EGCG is the most abundant catechin derived from green tea, which effectively improved hyperglycaemia and hyperinsulinaemia in high-fat, high-fructose or STZ-induced mice. The results strongly supported that the administration (~300 mg/kg) and intraperitoneal injection (25 and 75 mg/kg) of EGCG with the duration of 5 weeks to 17 weeks prevented liver IR or decreased the value of homeostatic model assessment of IR (HOMA-IR) in diabetic mice [41,42]. EGCG enhanced the protein expression of Nrf2 by 1.6-fold in comparison with high fat plus fructose-fed C57BL/6J male mice [27]. Protein tyrosine phosphatase 1B (PTP1B), as a negative regulator of glucose and insulin homeostasis, was decreased by EGCG treatment at the protein level [27]. The insulin receptor substrate binds to and activates PI3K, which is the key step in modulating glucose transport. The activation of PI3K by Fu brick tea extract, of which EGCG is the major active component, is the key step to modulate glucose transport. AKT was subsequently enhanced by EGCG, as it affects insulin function in glucose metabolism [27]. The phosphorylation of glycogen synthase kinase (GSK)3β and AMPK was further enhanced with EGCG supplementation. This significantly ameliorated IR through the
IRS-1/AKT/GSK3β/GLUT2 and Keap1/Nrf2 signaling pathways in liver tissues, resulting in increased protein expression levels of IRS-1 (Tyr 612), AKT, GLUT2, Keap1, and Nrf2 [27].

Pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are able to induce phase II detoxifying genes in response to exotic compounds. EGCG treatment significantly decreased the value of HOMA-IR from 3.78 to 2.13 [41]. EGCG evoked the protein expression of Nrf2 and its downstream targets HO-1 and NQO-1. Moreover, EGCG inhibited gluconeogenesis by decreasing the protein expression of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphate and lipogenesis by restoring the gene and protein expression of SREBP-1C, fatty acid synthesis (FAS), and acetyl-CoA carboxylase 1 (ACC1) in liver tissues. This effect was accompanied by enhancement of PXR/CAR-mediated phase II drug metabolism enzymes, including the gene and protein expression of sulfotransferases 1A1 (SULT1A1) and UDP-glucuronosyl-transferases 1A1 (UGT1A1) in the small intestine and liver [41]. However, the expressions of Keap1 under EGCG supplementation were different, which might be resulted from the experimental conditions, mouse type, duration, etc.

4.2 Effect of resveratrol in a diabetic animal model

Resveratrol is a stilbenoid compound produced by plants such as grapes and blueberries. The dose used in diabetes by oral administration ranged from ~2.5 to 600 mg/kg with a duration from 1 week to 26 weeks [43–50]. Resveratrol restored peripheral insulin sensitivity and hepatic insulin signaling in IRS-deficient mice and STZ-induced diabetic mice by decreasing the level of PTP1B in a sirt1-independent manner [46]. The possible additional mechanism might be through the Keap1-Nrf2 antioxidant system with the gene enhancement of GPX, HO-1, NQO1, and glutamate cysteine ligase modifier subunit (GCLM) [46]. Prefeeding with resveratrol stimulated the gene expression of Nrf2 by approximately 1.2 folds compared with that of the control and enhanced the gene expression of NQO1, and all these factors may have
contributed to protection from PCB-77-induced impairment of insulin signaling in adipose tissues [47]. An insulin tolerance test was used to evaluate insulin sensitivity in liver, muscle and adipose tissues, which was lowered by resveratrol, and the marked recovery of hepatic p-IRS (Tyr) by resveratrol treatment was observed at a dose of 10 mg/kg [48]. In a study conducted by fructose-induced male and female Wistar rats, cafeteria feeding of resveratrol had no effects on the increased IRβ, IRS-1 and IRS-2 mRNA expression in both male and female rats, while there was a significant decrease in PI3K in sexes as well as AKT, eNOS, and PPARγ in females. This indicated that the modulation of the insulin signaling pathway by resveratrol may be associated with inducers, animals, and sex [49]. *Graptopetalum paraguayense* ethanol extract, in which the active ingredient was gallic acid, increased insulin synthesis by increasing PPARγ and pancreatic-duodenal homeobox-1 (PDX-1) but inhibited the expression of CCAAT/enhancer binding protein-β (C/EBPβ) [50]. Additionally, the activation of Nrf2 strongly improved insulin sensitivity in the liver and muscle [50].

4.3 Effect of curcumin in the diabetic animal model

The polyphenol curcumin is isolated from the *Curcuma longa* plant. Curcumin improved glucose tolerance and insulin-stimulated PKB phosphorylation in muscles and live tissues and prevented Keap1 from targeting Nrf2 for ubiquitination and degradation, thus activating Nrf2 polyubiquitination and inhibiting the expression of Keap1 and proinflammatory cytokines, particularly through p38-mediated signaling pathways [29].

It is reasonable to believe that in the condition of nutrient oversupply, mitochondrial oxidative phosphorylation metabolism could produce a larger amount of ROSs. If antioxidative machinery cannot eliminate mitochondrial-derived oxidants, oxidative stress may occur, resulting in IR [51]. In addition to the preventive effect of curcumin on IR, we demonstrated here that after the induction of obesity by a high-fat diet, short-term curcumin
gavage still reversed glucose intolerance, clearly suggesting the therapeutic application of curcumin in the treatment of IR-related metabolic disorders [51–53]. In accordance, our study provided a significant mechanism of Nrf2 activation by which curcumin can defend against oxidative stress and mitochondrial redox imbalance and attenuate the abnormality of glucose metabolism in the condition of nutritional oversupply. Oral gavage of curcumin decreased HOME-IR and restored or enhanced the protein expression of Nrf2 in the muscle and liver tissue of mice [29,52]. The protein expression levels of nuclear p65 and Keap1 were decreased when treated with curcumin [29]. The novel curcumin C66 protected against diabetes-induced aortic damage, which was associated with the suppression of JNK2 expression [53]. However, whether curcumin inhibits proteasomal degradation or acts specifically on Nrf2 proteins is still unknown [29]. Since mitochondrial oxidative stress plays a major causative role in IR in the condition of nutrition overconsumption, glycyrrhizin supplementation upregulated the Nrf2/HO-1 oxidative signaling pathway and suppressed the expression of gluconeogenic enzymes [51]. Curcumin prevented oxidative and endoplasmic reticulum stress by the activation of the Nrf2/HO-1 pathway and the inhibition of NF-κB signaling through PI3K/AKT, along with the antiapoptotic signaling cascade involved in diabetic rats [54].

4.4 Other natural phytochemicals in the diabetic animal model

While EGCG, resveratrol and curcumin are the most natural product inducers of the Nrf2 signaling pathway in IR and diabetes in animal models, there are many other natural sources that can activate the pathway and therefore prevent or treat T2D. Fu brick tea aqueous extract (FTE) at a dose of 400 mg/kg in rats significantly downregulated SIRPα expression and activated the insulin signaling AKT/GLUT4, FoxO1, mTOR/S6K1 pathways in skeletal muscle [55]. A high dose of aspalanthin (130 mg/kg) activated the Nrf2 pathway in db/db
mice and upregulated the mRNA expression of GPX2, glutathione synthetase (GSS) and homo sapiens parkinson protein 7 (PARK7), with a decrease in caspase-3 and NADPH oxidase 4 (NOX4), indicating a preventive effect of high glucose-associated complications [56]. Ginsenoside Rg1, as a major active ingredient in processed ginseng, promoted insulin secretion, weakened the function of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3), and subsequently upregulated the Nrf2/ARE pathway, following the increased production of antioxidant enzymes [57]. Phloretin prevented diabetic cardiomyopathy by dissociating the Keap1/Nrf2 complex, and this compound might directly bind Keap1, resulting in the promotion of Nrf2 expression [58]. Hedansanqi Tang et al. [59] extract prevented nonalcoholic fatty liver disease in vivo by promoting lipolysis in 3T3-L1 adipocytes and activating the Nrf2/HO-1 antioxidant signaling pathway in the liver. Similarly, pterostilbene enhanced the production of Nrf2 in rats and mice [60]. Compound Centella, an herbal complex, presented protective effects against diabetic kidney disease, which may also be associated with the Keap1/Nrf2-ARE pathway under oxidative stress, but its specific antioxidant ingredients remain unknown [61]. Dark tea extracts could also activate the PI3K/Akt-PPAR signaling pathway to regulate blood glucose and IR, change the key enzyme activities related to glucose metabolism and antioxidant activity, and reduce oxidative stress and inflammatory factor levels in T2D [62]. Treatment of diabetic rats with the ethanolic extract of Chromolaena odorata leaves (200 mg/kg), however, upregulated the mRNA expression of Glut2, glucokinase and Nrf2 but repressed Keap1 mRNA expression. Docking results showed that 5,7-dihydroxy-6 – 4-dimethoxyflavanone and luteolin, as possible compounds from Chromolaena odorata, potentiated this protective role [63]. Oral administration of naringenin enhanced the protein expression of Nrf2 and thereby increased the activities of SOD, CAT, glutathione peroxidase (GPX), GST enzymes and the levels of GSH in the pancreatic tissues of STZ-treated mice [31]. Chrysophyllum albidum fruit pulp
powder (CAFPP) significantly upregulated the antioxidant genes Nrf2 (approximately 2 folds) and CAT, GST, SOD, and insulin regulation-related genes in the diabetic group (P<0.05) compared to the diabetic control with concomitant downregulation of TNF-α and DPP4 gene expressions [64]. Berberine is a promising antidiabetic isoquinoline alkaloid that is converted into the absorbed metabolite oxyberberine, both of which can exist in blood in a protein-bound form. Berberine possessed protective effects on hypoglycemic and β-cells through gut microbiota modulation and the PI3K/AKT and Nrf2 pathways activation [65]. Almond oil treatment inhibited oxidative stress and inflammatory reactions, enhanced liver and kidney function, and upregulated the protein expression of Nrf2, HO-1, and NQO1 but downregulated the expression of Keap1 [66]. It also reversed the gut microbiota changes induced by STZ and this effect was associated with glucose metabolism [66]. However, the current literature on the crosslink of phytochemicals targeting the Nrf2 pathway and gut microbiota is rare, and the Nrf2 response in liver and muscle and its relationship with the Nrf2 response in the gut and its associated liver-gut axis changes need to be further investigated in the future.

5 Phytochemical Nrf2 Activators Targeted Diabetes in Human Clinical Trials

T2D shows the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) [67]. These patients have an increased risk of developing many complications, such as diabetic retinopathy with progression of the disease leading to blindness and end-stage renal failure [68], cardiovascular disease (CVD) leading to atherosclerosis [69], diabetic nephropathy leading to scarring changes in the kidney tissue, loss of small or progressively larger amounts of proteins in the urine, and eventually chronic kidney disease requiring dialysis [16]. Excess oxidative stress plays a causal role in the development and progression of the above diabetic complications through a variety of
mechanisms, with increased levels of ROSs and mitochondrial dysfunction [70].

Several natural Nrf2 activators have been or are currently being tested in clinical trials of T2D (Table 3). Dose and duration are of great importance in the clinical study of the function of phytochemicals. Curcumin (500 mg/day) can reduce U-mAlb excretion in T2D patients, enhance the Nrf2 system and specifically regulate NAD(P)H quinone oxidoreductase 1 and other antioxidative enzymes, including SOD1 and SOD2 [71]. The levels of MDA and LPS were dramatically reduced by approximately 75% and 25%, respectively [71]. The reduction in LPS was associated with the enhancement of gut bacteria for maintaining gut microbiota balance and gut barrier function, including Bacteroides, Bifidobacterium and Lactobacillus [71]. The inflammatory inhibitory protein IκB was clearly increased after curcumin treatment, indicating that the cellular damage with inflammation-initiated apoptotic processes resulting from oxidative stress was alleviated by curcumin [71]. However, there was no significant difference observed in fasting blood glucose, C-peptide, TC, TG, HDL-C, LDL-C and insulin levels after curcumin intake [71]. For resveratrol consumption (800 mg/day), changes in plasma glucose, HbA1c, insulin and HOMA-IR levels were not significant, while higher levels of TC and LDL-C were obtained, possibly because different lipid-lowering drugs were taken by patients [72]. The transcription of HO-1, NOS, RAGE and CAT did not change with resveratrol supplementation, while the transcript levels of Nrf2 and SOD were significantly increased [72]. There was no significant difference in hydrogen peroxide (H$_2$O$_2$) between the resveratrol and placebo groups, whereas patients taking resveratrol had significantly lower intracellular superoxide anion (O$_2^{-}$) production after receiving resveratrol for 2 months [72]. These natural-origin activators were found to improve insulin sensitivity, increase the total antioxidant capacity, improve lipid profiles, lower fasting glucose levels and reduce inflammation levels [73].

In addition, ninety Nrf2 activators have been performed in clinical trials together in
NCBI (https://clinicaltrials.gov/ct2/results?cond=&term=Nrf2&cntry=&state=&city=&dist=). Among them, approximately fourteen Nrf2 phytochemical activators are focused on diabetes prevention or treatment. However, the final successful application in human clinical is to be continued.

6. Precision Nutrition, Nrf2-ARE Signaling Pathway and Diabetes

Nutrigenomics refers to the use of biochemistry, physiology, nutrition, genomics, proteomics, metabolomics, transcriptomics, and epigenomics to seek and explain the existing reciprocal interactions between genes and nutrients, including bioactive compounds, at the molecular level [74]. Individual nutrition with multiomics technologies contributes to precision nutrition and reduces the prevalence of T2D [75,76]. Four key genes (KCNJ11, PPAR-γ, TCF2, and WFS1) were originally identified related to T2D, of which the PPAR-γ variant genotype is associated with different types and levels of lipids [77]. PPAR-γ in adipose tissue, glucocorticoid receptor and FoxO1 in insulin-sensitive tissues in combination with PGC-1α and CRTC2, and chromatin-modifying enzymes, including DNA methyltransferases and histone acetyltransferases, which regulate a variety of key genes for the control of IR [78]. Specific expressions of CBX8, DDA1, PIK3R6, GATM and WDR41 were identified in diabetic mice by genome-scale transcriptional analysis [79]. Bioinformatics identified that CD8A and CCL5 were associated with T2D, proving it as unique target genes for understanding its mechanism; hence, these genes might act as potential biomarkers for personalized therapies for diabetes at an early stage [80]. Polyphenols such as resveratrol, quercetin, catechins, curcumin and dihydromyricetin, which are abundant secondary metabolites in plants, were reported to play a key role in the prevention and treatment of diabetes, as the related genes or protein expressions were activated or inhibited [75,81]. Thus, it is important to understand the targets of T2D from the perspective of nutrigenomics and
then explore the role and mechanism of phytochemicals.

Our research team plans to perform further research by using nutrigenomics and precision nutrition to elucidate the crucial role of the Nrf2-ARE pathway in the modulation of other redox signaling pathways, especially the IRS-1, IGF-1, PPAR, AMPK, and autophagy/apoptosis pathways.

7. Interactions among the Microbiota Interface, Nrf2-ARE Signaling Pathway and Diabetes

The gut microbiota of diabetic patients is known to differ from that of healthy individuals, and gut microbiota disorder refers to the onset and maintenance of T2D [82]. The dysbiosis of gut microbiota showed higher endotoxemia and dysfunction of the intestinal barrier, with the alteration of the gut-liver axis, subsequently leading to an inflammatory response [83]. Dietary interventions have been successful in altering the abundance, composition, and activity of gut microbiota that are relevant for food metabolism and glycemic control, providing compelling evidence for the prevention and treatment of diabetes. In addition, mobile apps and wearable devices facilitate real-time assessment of dietary intake and provide timely feedback, contributing to glycemic control and T2D management [84]. The administration of quercetin reversed gut microbiota imbalance and related endotoxemia-mediated TLR-4 pathway induction, which was accompanied by the inhibition of the inflammasome response and ER stress pathway activation, leading to the blockage of lipid metabolism gene expression deregulation [83]. Dihydromyricetin significantly increased the beneficial Lactobacillus and Akkermansia genera in a mouse model of inflammatory bowel disease, and this is correlated with the increased gastrointestinal levels of unconjugated bile acid, which could activate specific receptors, such as FXR and TGR5, and maintain intestinal integrity [85]. The modulatory effects of vine tea on metabolic syndrome were found to target
redox balance, including the Nrf2, NF-κB, PI3K/IRS2/AKT, AMPK-PGC1α-SIRT1, and SIRT3 pathways and gut microbiota, by upregulating the ratio of Firmicutes/Bacteroidetes (F/B) and increasing the relative abundance of Akkermansia muciniphila [81]. Fuzhuan brick tea is beneficial for the gut microbiota composition and structure in HFD-induced mice, while the metabolism of caffeine is also crucial in the process [86,87]. Instant dark tea had a significant impact on the gene expression of biomarkers in lipogenesis in the liver and modified the gut microbiota species (significantly enhanced Akkermansia) in HFD-induced mice, of which Ruminococcus 1 was found to be a potential biomarker that was strongly correlated with oxidative stress (including Nrf2) and metabolism genes [88].

Our research team plans to further elucidate the crucial role of the Nrf2-ARE pathway in the modulation of gut microbiota, especially on probiotics, harmful bacteria and diabetic or diabetes-related bacteria. In addition, we will also focus on the relations among intestinal ROS levels, microbiota and diabetes, and we wish to elucidate the molecular mechanism by targeting the Nrf2 pathway to extinguish intestinal ROSs for the chemoprevention of diabetes.

8. Conclusions and Prospective

This review summarizes recent research progress of phytochemicals targeting T2D by the Nrf2 signaling pathway in recent years in vitro and in vivo, and the related molecular mechanism and pathways were shown in Figure 3, which may pave the way for more phytochemicals to the attending of clinicians. EGCG, resveratrol and curcumin are considered as the most common natural activators of T2D and inhibit or modify the structure of Keap1 protein, suppress the proteasomal degradation of Nrf2, modulate the AKT/GSK-3 pathway, promote antioxidant enzymes and cross-link with anti-inflammatory pathways such as NF-κB. Recent findings in phytochemicals in animal and clinical studies have given us insights into the molecular mechanism of Nrf2 pathway activation in diabetes and the
potential connection with the gut-liver axis, highlighting important therapeutic targets for future chronic diseases.

Future investigations of dose–responses of biomarkers will provide insights into associations between possible mechanisms of action and clinical outcomes [89]. Comprehensive evaluation is urgently needed for the dual role of Keap1-Nrf2-ARE pathway activation; in particular, the sustained activation of Nrf2 appears to favor the progression of cancer and other diseases. Thus, the utilization of artificial intelligence techniques combined with nutrigenomics may help us understand the precision nutrition of phytochemicals targeting the Nrf2 pathway. In addition, most of the in vitro studies ignored the digestion and adsorption of phytochemicals, which will be important factors for the further utilization of these components in food and medicine. The gut microbiota is highly associated with T2D, and the role of phytochemicals in the redox interface and balance in the intestinal barrier and their relationship with ROS extinction, as well as the crosstalk among multiple cellular signaling pathways, including sn-1,2-DAG/PKC/IRS, Nrf2/NF-κB, PPARs, AMPKs, mechanistic target of rapamycin (mTOR), and insulin growth factor-1 (IGF-1), are worth further investigation. These phytochemicals could shape the gut microbiota structure to some extent in animal models and clinical trials, for example, enhancing the relative abundance of star microbiota Akkermansia. However, further understanding of the evolutorial time and space changes of the gut microbiota by phytochemicals related to the Nrf2/ARE signaling pathway by integration of multiomics is of great importance, especially for clinical research and applications in the future.

**Funding**

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Postdoctoral Project (No. 2021RC2080).

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**Figure legends**

**Figure 1. Nrf2 activation by phytochemicals in preventing diabetes** Excessive oxidative stress and prolonged exposure to ROS are the underlying factors associated with mitochondrial dysfunction and hyperglycemia, resulting in diabetes. The activation of Nrf2 signaling pathway by various phytochemicals enhanced the amount of phase II enzymes, inhibited the level of ROS and protected mitochondrial and β cell functions.

**Figure 2. The mechanism of activation of the Keap1-Nrf2 signaling pathway** Under homeostatic conditions, Nrf2 is kept at an off position bound to endogenous Keap1, which functions as an E3 ubiquitin ligase and constantly targets Nrf2 with Cul3-Rbx1 for ubiquitination and proteasomal degradation. In the case of oxidative stress, Nrf2 detaches from Keap1 and translocates to the nucleus, where it heterodimerizes with Maf, and heterodimers recognize and bind to antioxidant response elements (AREs), affecting the expression of a variety of genes, such as antioxidant enzymes and cytokines.

**Figure 3. Molecular mechanism and signaling pathways of phytochemicals against diabetes** Phytochemicals regulate diabetes mainly through glucose and lipid metabolism pathways and redox balance pathways: increase glucose transporter 4 (GLUT4) and GLUT2 translocation and activate phosphatidylinositol 3-kinase (PI3K)-Akt pathways to enhance glucose uptake and prevent insulin resistance; phytochemicals inhibit gluconeogenesis by decreasing the protein expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphate (G-6-pase); phytochemicals increase antioxidant enzymes by activating Nrf2 signaling, then inhibit level of ROS and NF-κB pathway; phytochemicals restore the expression of SREBP-1C, fatty acid synthesis (FAS), and acetyl-CoA carboxylase 1 (ACC1) to inhibit lipogenesis.
<table>
<thead>
<tr>
<th>Phytochemical type</th>
<th>Model</th>
<th>Dose</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenethyl isothiocyanate</td>
<td>( \text{H}_2\text{O}_2 ) induced 3T3-L1 adipocytes</td>
<td>15 µM</td>
<td>↑Nrf2, γ-GCS, HO-1, NQO1, GST, and GLUT4</td>
<td>[23]</td>
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<tr>
<td>Dihydrocurcumin</td>
<td>Oleic acid induced L02 and HepG2 cells</td>
<td>5–50 µM</td>
<td>↑pAKT, PI3K, PPARα, and Nrf2</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓TG, NO, and ROS</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>↑p-IRS-1 Ser307, and ROS</td>
<td></td>
</tr>
<tr>
<td>Tartary buckwheat flavonoids</td>
<td>High-glucose induced HepG2 cells</td>
<td>25–100 µg/mL</td>
<td>↑Nrf2, IRS-1, and GSH</td>
<td>[25]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>↑p-IRS-1 Ser307, and ROS</td>
<td></td>
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<tr>
<td>Ellagic acid</td>
<td>High glucose induced HepG2 cells</td>
<td>15, 30 µM</td>
<td>↑miR-223, Nrf2, HO-1, SOD1, SOD2, p-IRS1, p-AKT, and p-ERK</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑Keap1, and ROS</td>
<td></td>
</tr>
<tr>
<td>EGCG</td>
<td>Glucosamine induced HepG2 cells</td>
<td>0–50 µM</td>
<td>↑PI3K/AKT, Nrf2, HO-1, NQO-1, and IRS-1/AKT/GLUT2</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑PTP1B</td>
<td></td>
</tr>
<tr>
<td>EGCG</td>
<td>Palmitic acid induced HepG2 cells</td>
<td>50 µM</td>
<td>↑SOD, SOD2, glutathione peroxidase, GLUT2, (PGC)-1b, and SREBP-1c</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ROS</td>
<td></td>
</tr>
<tr>
<td>Curcumin</td>
<td>HepG2 cells</td>
<td>/</td>
<td>↑Nrf2</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑TNFα, p38, and Keap1,</td>
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<tr>
<td>Compound</td>
<td>Condition</td>
<td>Concentration</td>
<td>Effect</td>
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<tr>
<td>Benzyl Isothiocyanate</td>
<td>Palmitic acid induced C2C12 cells</td>
<td>2.5-5 μM</td>
<td>↑Nrf2, HO-1, GSTP, GCLM, GSH, p-IRS-1, p-AKT, p-TBC1D1, GLUT4, PPARγ, and EBPα ↓ROS</td>
<td>[30]</td>
</tr>
<tr>
<td>Naringenin</td>
<td>STZ-induced MIN6 cells</td>
<td>25, 50, 100 μM</td>
<td>↑Nrf2, NQO1, and GST</td>
<td>[31]</td>
</tr>
<tr>
<td>Vitexin</td>
<td>High glucose-induced INS-1 β-cells</td>
<td>20, 40 μM</td>
<td>↑Nrf2, NQO1, GST, SOD, CAT, GPX, GSH, IRS-2, and GLUT2 ↓p65, IκB, RelB</td>
<td>[32]</td>
</tr>
<tr>
<td>Aspalathin (apigenin-8-C-glucoside)</td>
<td>INS-1E cells</td>
<td>30, 60 μM</td>
<td>↑ Nrf2, Hmox2, NQO1, and SOD1</td>
<td>[33]</td>
</tr>
<tr>
<td>Morin</td>
<td>STZ and H₂O₂ induced INS-1E cells</td>
<td>5, 10 μM</td>
<td>↑ Nrf2, pNrf2, NQO1, GST, SOD, CAT, and GPX</td>
<td>[34]</td>
</tr>
<tr>
<td>Silibinin</td>
<td>Oleic acid induced HepG2 cells</td>
<td>5–100 μM</td>
<td>↑CFLAR, PPARα, PI3K, pAKT, and NRF2 ↓TG, NO, SREBP-1C, PNPLA3, p-JNK, CYP 2E1 and CYP 4A</td>
<td>[35]</td>
</tr>
<tr>
<td>Daphnetin</td>
<td>Oleic acid induced HepG2 cells</td>
<td>5–50 μM</td>
<td>↑phosphorylation of AMPK, PI3K, p-AKT, and Nrf2 ↓SREBP-1C, PNPLA3, CYP2E1, CYP4A, and ROS</td>
<td>[36]</td>
</tr>
<tr>
<td>C-glycosides</td>
<td>High-glucose induced L02 and HepG2 cells</td>
<td>100–200 μg/mL</td>
<td>↑p-JNK, p-p38, Nrf2, HO-1, NQO1, PGC-1α, and FoxO1</td>
<td>[37]</td>
</tr>
</tbody>
</table>
Table 2. Role of phytochemicals on diabetes by targeting Nrf2 signaling pathway in animal model

<table>
<thead>
<tr>
<th>Phytochemical type</th>
<th>Animal model</th>
<th>Dose/duration</th>
<th>Administration</th>
<th>Molecular target</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>HFD and high-fructose fed C57BL/6J male mice</td>
<td>~255 mg/kg, 16 weeks</td>
<td>Drinking water feeding</td>
<td>↑Nrf2, Keap1, HO-1, NQO1, IRS-1(Tyr 612), AKT, GLUT2, p-AMPK, p-GSK3β, and p-ACC</td>
<td>↓PTP1B, SREBP-1, and FAS</td>
<td>Prevent liver insulin resistance; Increase liver GPX1, CAT, MnSOD, GSH, GST, GPX; Decrease liver MDA</td>
</tr>
<tr>
<td>EGCG</td>
<td>STZ induced and high fat induced male ICR mice</td>
<td>300 mg/kg, 5 weeks</td>
<td>Oral administration</td>
<td>↑PXR, CAR, SULT1A1, UGT1A1, SULT1A1, SSULT2B1b, Nrf2, CREB, and PGC1α</td>
<td>↓HOMA-IR, Keap1, SREBP-1c, FAS, ACC1, PEPCK, G-6-Pase, CD36, PPARγ, and HOMA-IR</td>
<td>No difference on body weight; Improved homeostasis, phase II drug metabolism enzymes expression; Decrease liver weight, LDL, and TG</td>
</tr>
<tr>
<td>EGCG</td>
<td>HFD induced male C57BL/6J mice</td>
<td>25, 75 mg/kg, 17 weeks, three times/week</td>
<td>Intraperitoneal injection</td>
<td>↑Nrf2, and HO-1</td>
<td>↓RAGE, liver and plasma AGEs, and HOMA-IR</td>
<td>Decrease body weight gain, liver weight, kidney weight, serum glucose and insulin, ALT, AST; Increase GSH, GSH/GSSG; Alleviate diabetes complications</td>
</tr>
<tr>
<td>Curcumin</td>
<td>HFD induced C57BL/6J male mice</td>
<td>50 mg/kg, 10 days</td>
<td>Oral gavage</td>
<td>↑IκBα, p-PKC, total Nrf2, NQO1</td>
<td>↓nuclear p65, and Keap1</td>
<td>Decrease blood glucose; Improved glucose tolerance; Decrease mitochondrial MDA; Increase GSH</td>
</tr>
<tr>
<td>Curcumin</td>
<td>HFD induced male C57BL/6J mice</td>
<td>50 mg/kg, 15 days</td>
<td>Oral gavage</td>
<td>↑Skeletal muscle nuclear, and HO-1</td>
<td></td>
<td>Improve glucose intolerance; Decrease skeletal muscle MDA</td>
</tr>
<tr>
<td>Treatment</td>
<td>Model</td>
<td>Dose</td>
<td>Route</td>
<td>Effect on Markers/Parameters</td>
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<tr>
<td>Curcumin</td>
<td>Diabetic rats</td>
<td>100 mg/kg•BW, 8 weeks</td>
<td>Oral gavage</td>
<td>↑HOMA-IR, hepatic and TNF-α, ↑Nrf2, HO-1, ↑NF-κB, Fas-R, and cleaved Caspase-8, ↑Weight of the pancreas, plasma insulin and mitigated oxidative stress related markers; ↓Blood glucose level</td>
<td></td>
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</tr>
<tr>
<td>Naringenin</td>
<td>STZ-induced</td>
<td>50, 100 mg/kg, 45 days</td>
<td>Intragastric</td>
<td>↑Nrf2, SOD, CAT, GPX, GST, GSH, and TBARS, Decrease blood glucose, glucose-6-phosphatase, fructose-1,6-bisphosphatase; Increase insulin secretion, hexokinase, glucose-6-phosphate dehydrogenase, glycogen; Normalize lipid profile, restoration of insulin expression; Promote glycolysis; Inhibit gluconeogenesis</td>
<td></td>
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</tr>
<tr>
<td>Resveratrol</td>
<td>HFD induced C57BL/6J mice</td>
<td>0.06%, 26 weeks</td>
<td>Pelleted diet</td>
<td>↑Nrf2, HO-1, NQO-1, CAT, SIRT1, GLUT4, AKT, and PI3K, ↑GPX, Hmox1, NQO1, GCLM, pIRβ (Tyr1163), pIRS1(Tyr1179), and pAKT (Thr308, Ser473), ↓PTP1B, and Ptpn1, Decrease the weight of adipose, plasma leptin; cholesterol, TG, LDL-C, fasting blood glucose, and plasma insulin; Increase HDL-C, GSH/GSSG, T-AOC, and Tregs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>IRS−/− mice</td>
<td>~2.5 mg/kg, 8 weeks</td>
<td>Drinking water feeding</td>
<td>↑GPX, Hmox1, NQO1, GCLM, pIRβ (Tyr1163), pIRS1(Tyr1179), and pAKT (Thr308, Ser473), ↓PTP1B, and Ptpn1, No significant change on serum glucose and insulin; Alleviate severe insulin resistance; Unable to overcome the beta cell failure; restore the hepatic insulin signaling and trigger the antioxidant response; Recover skeletal muscle insulin signaling; Increase peripheral insulin independently of Sirt1 and associated with inhibition of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Model Description</td>
<td>Dose/Course</td>
<td>Administration</td>
<td>Tissue/Cytokine</td>
<td>Outcome</td>
<td>Reference</td>
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</tr>
<tr>
<td>Resveratrol</td>
<td>PCB-77 induced male C57BL/6J mice</td>
<td>160 mg/kg, 1 week</td>
<td>Pre-fed diet</td>
<td>Adipose tissue ↑Nrf2, NQO1, and p-AKT</td>
<td>Decrease blood glucose and insulin tolerance test</td>
<td>[47]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Methyglyoxal (MG)-induced male Balb/C mice</td>
<td>10 mg/kg, 12 weeks</td>
<td>Oral gavage</td>
<td>↑p-Nrf2, and IRS-Tyr phosphorylation [HOMA-IR, and hepatic TNF-α]</td>
<td>Decrease blood glucose, insulin tolerance test; Increase pancreatic insulin level</td>
<td>[48]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Fructose-induced male and female Wistar rats</td>
<td>500 mg/kg, 24 weeks</td>
<td>Cafeteria feeding</td>
<td>Adipose tissue ↑PI3K, Nrf2, iNOS, IL-6, IL-10, and IL-18 Male: MDA, IL6, IL-10, and IL-18 Female: AKT, PPARγ, TNF-α, ALT, and eNOS</td>
<td>↓Adipose insulin, MDA, AST, and TG; No effects on IRβ, IRS-1, IRS2, and SIRT1</td>
<td>[49]</td>
</tr>
<tr>
<td>Resveratol/Gravitopetalum paraguayense ethanol extract</td>
<td>CML (Carboxymethyllysine) induced C57BL/6J mice</td>
<td>10 mg/kg, 12 weeks/10 mg/kg, 12 weeks/300 mg/kg, 12 weeks</td>
<td>Intraperitoneal injection</td>
<td>↑PPARγ, PDX-1(pancreatic-duodenal homeobox-1), p-Nrf2, GCL (glutamate-cysteine ligase), GSH, liver and muscle p-AKT ↓C/EBPβ, and GSIS</td>
<td>Decrease blood glucose, plasma insulin; Protect against pancreas damage and elevate islet numbers; Increase pancreas insulin expression and GCL expression; Increase 2-NBDG uptake in liver and muscle; Decrease hepatic MDA</td>
<td>[50]</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>HFD induced male Wistar rats</td>
<td>50 mg/kg, 30 days</td>
<td>Oral gavage</td>
<td>↑TAC, liver Nrf2, nuclear Nrf2, and HO-1 ↑HOMA-IR, and hepatic TNF-α</td>
<td>Decrease rat weights and insulin resistance; Reduce adipocytes size in adipose and lipid deposition in liver tissue; Decrease TC, TG, LDL-C, and liver MDA; Increase insulin sensitivity; Suppress gluconeogenesis</td>
<td>[51]</td>
</tr>
<tr>
<td>Phytochemical</td>
<td>Dose/Medication/Duration</td>
<td>Subjects</td>
<td>Number and gender</td>
<td>Age range</td>
<td>Effect or biomarker</td>
<td>Mechanism</td>
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</tr>
<tr>
<td>Curcumin</td>
<td>500 mg/day, powder, 15–30 days</td>
<td>Type II diabetic patients</td>
<td>14 (F=6, M=8)</td>
<td>47–85</td>
<td>(\uparrow)Bifidobacterium, Lactobacillus, Bacteroides (\downarrow)U-mAlb excretion, level of MDA and LPS in blood</td>
<td>(\uparrow)Nrf2, NQO1, SOD1, SOD2, and IkBα (\downarrow)Caspase 3</td>
</tr>
</tbody>
</table>

HFD: High-fat diet; STZ: Streptozotocin

Table 3. Role of phytochemicals on diabetes by targeting Nrf2 signaling pathway in clinical research
| Resveratrol | 800 mg/day, capsules, 2 months | Type II diabetic patients | 41 (F=19, M=22) | 30–70 | ↑Total antioxidant capacity and protein thiol content of plasma; ↓Plasma protein carbonyl content | ↑Nrf2 and SOD ↓intracellular superoxide anion (O$_2^-$) |

[72]
Mitochondrial dysfunction -> ROS -> Diabetes (Insulin resistance, β cell dysfunction)

Phytochemicals -> Nrf2 activation -> Induction of phase II enzymes
Cytoplasm

Under homeostatic condition

Proteosome

Transcription of target genes
- Antioxidant enzymes (HO-1, NQO1, SOD, CAT, GSH, etc)
- ROS, Cytokines production

Under oxidative stress

74x30mm (300 x 300 DPI)
NRF2-ARE activation

- Increase glucose uptake
- Inhibit gluconeogenesis
- Prevent insulin resistance
- Inhibit lipogenesis
- Increase antioxidant enzymes
- Inhibit ROS and inflammation

<table>
<thead>
<tr>
<th>Mitochondrial function</th>
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</table>

| β cell function |

338x190mm (95 x 95 DPI)
Insulin resistance (IR) and mitochondrial dysfunction are fundamental to the development of type 2 diabetes (T2D). Here, we discuss that phytochemicals prevent and treat T2D via activation of the Nrf2 signalling pathway.

- EGCG, resveratrol and curcumin are considered as the most potent natural Nrf2 activators against diabetes.
- Upregulation of Nrf2 by phytochemicals is correlated with the elimination of excess ROS and increased induction of antioxidant enzymes as well as phase II drug metabolism enzymes in T2D.
- Nrf2 activation by phytochemicals plays a critical role in maintaining redox balance and regulating glucolipid metabolism via the GLUT4/IRS1/PI3K/AKT and SREBP1C/FAS/ACC1 pathways in T2D.