3. REVIEW OF LITERATURE

a. Role of Nrf2 in modulating metabolic aberrations during hyperglycemia

Hyperglycemia arising from uncontrolled glucose regulation cause tissue damage through (I) increased polyol pathway flux; (II) increased advanced glycation end product (AGE) formation; (III) activation of protein kinase C (PKC) isoforms (10). All these consequences lead to the over-production of reactive oxygen species (ROS), thereby create an oxidative environment, which ultimately leads to the development of insulin resistance, impaired glucose tolerance and β-cell dysfunction (82, 83). Several lines of evidence indicate that Nrf2 plays an important role in modulating metabolic signaling cascades in such a way to promote the utilization of glucose while reducing pathways involved in the production of glucose molecules (24). Therefore, targeted activation of Nrf2 using naturally occurring phytochemicals such as resveratrol and curcumin is health beneficial as they help in mitigating diabetes and its complications (50). However, due to poor solubility, bioavailability and toxic systemic effects at doses required for better efficacy, the utility of resveratrol and curcumin have been discouraged, warranting the identification of more potent, soluble and bioavailable compounds for activating Nrf2 in diabetes (84, 85). In the subsequent sections of literature review, first, the emphasis is given to address the role of Nrf2 in regulating various metabolic pathways associated with diabetes, followed by an account on naturally occurring Nrf2 activators. Furthermore, key areas that require more in-depth research are also mentioned.

(I) Role of Nrf2 in polyol pathway: Aldose reductase (AR), a member of the aldo-keto reductase protein family, induces the deposition of sorbitol causing increased levels of oxidative stress (86). Sorbitol, a sugar alcohol, deposition is one of the risk factors associated with diabetes-induced retinopathy (87). Hence inhibiting AR or its upstream regulators such as TGFβ1 using pharmacological agents help to retard the complications of diabetes (88). One key upstream regulator of AR1 is TGFβ1 (89). TGFβ1 stimulates AR’s expression via MAPK signal pathway and transcription factor AP-1 (90). Therefore, targeted inhibition of TGFβ1 represses the expression of AR, thereby decrease the activity of polyol pathway (91). However, interestingly, it has been found that activation of TGFβ1 inhibits Nrf2 expression resulting in elevated ROS levels (91). The elevated ROS further activate the TGFβ1 pathway, leading to the increased expression of AR (92). Hence decreasing cellular ROS through Nrf2 activation helps to inhibit TGFβ1 and downstream AR1 (93). Supporting this, a study by Jiang et al. (2010) demonstrated that Nrf2 negatively regulates TGFβ1 (94). Mice lacking Nrf2 but not Nrf2+/+ mice expressed elevated TGFβ1 (94). Furthermore, the activation or overexpression of Nrf2 inhibited the promoter activity of TGFβ1, whereas knockdown of Nrf2 using siRNA enhanced TGFβ1 transcription (94). Although this study provides an indirect association between Nrf2 and AR/polyol pathway further mechanistic studies are warranted to establish this association (94).

(II) Role of Nrf2 in the metabolism of advanced glycation end-products (AGEs)

In hyperglycemia, advanced glycation end-products (AGEs) are formed as a consequence of non-enzymatic binding of reducing sugars with the amino groups in proteins, lipids or nucleic acids, which mainly includes toxic carbonyl precursors such as methylglyoxal (MG, an active AGEs precursor), glyoxal, and carboxymethyl lysine (95). AGEs thus formed causes damage to target cells by directly disrupting matrix-matrix and matrix-cell interactions through the induction of excessive cross-linking of proteins (96). Further, AGEs also interact with the specific receptor, RAGE, to generate the ROS and activate various intracellular signaling events, including PKC, MAPK, NfkB (96). To overcome these toxic effects, it is essential to increase the expression of glyoxalase-1 (Glo-1), which
catalyzes the conversion of MG to lactic acid, thereby maintain the overall anti-oxidant status in a balanced state (97, 98). Interestingly, Glo-1 is a direct target of Nrf2 (98). Recently, a study has shown that induction of Nrf2 increased hepatic glyoxalase mRNA and glutathione (GSH), which subsequently metabolized MG into D-lactate acid, thereby reduced not only the serum and hepatic AGEs levels but also the levels of inflammatory factors in MG-treated experimental mice (97). In addition, Nrf2 activation prevented the AGEs-induced ROS formation in LX-2 and human stellate cells, through the upregulation of γ-glutamyl cysteine synthetase and glutathione synthesis (Figure 5) (99). Similarly, in AGEs induced glomerular mesangial cells (GMCs) Nrf2 promoted the expressions of target genes, heme oxygenase 1, superoxide dismutase and thereby decreased ROS levels, fibronectin, and TGF-β1 expressions. In summary, Nrf2 activation helps in blocking the pathological progression of diabetic retinopathy (Figure 5) (100).

(III) Role of Nrf2 in inhibiting protein kinase-C and its downstream targets

Nrf2 plays a critical role in inhibiting the inflammatory responses mediated by protein kinase – C (101). Protein kinase C (PKC) is serine/threonine kinases responsible for different structural and functional changes that include cellular permeability, inflammation, cell growth, angiogenesis, extracellular matrix expansion, and apoptosis (83). PKC stimulates intracellular molecules such as eNOS, NADPH oxidase, endothelin-1 (ET-1), phospholipase A2 (PLA2), vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF), vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), NF-kB and TGF-α (83). Nrf2 protects eyes from diabetic retinopathy by inhibiting TNF-α, which otherwise cause the death of pericytes and endothelial cells (100). Diabetic mice deficient in Nrf2 showed elevated retinal TNF-α expression, leading to vascular leakage (100). Likewise, STZ induced diabetic mice lacking Nrf2−/− also exhibited more severe glomerular injury, together with higher ROS production and increased expression of the profibrotic cytokine, fibronectin and transforming growth factor-β (TGF-β) when compared with diabetic WT mice (94). These changes were accompanied by a significantly greater urinary albumin to creatinine ratio (UACR), strongly suggesting the critical role played by Nrf2 in inhibiting the inflammatory responses (94).

While Nrf2 knockout studies clearly demonstrated the role of Nrf2 in inhibiting PKC-mediated inflammatory responses, it is not fully known whether activating endogenous Nrf2 using pharmacological agents also provide a similar health benefit in combating inflammatory diseases (102). Addressing this, a study has demonstrated that Nrf2 activators sulforaphane and cinnamic aldehyde improved renal performance and reduced pathological alterations in the glomerulus of STZ-Nrf2+/− mice compared to the Nrf2−/− mice (103). Nrf2 activation reduced oxidative damage and inhibited the expression of TGF-β1, extracellular matrix proteins and p21 (103). Similarly, curcumin, a potent Nrf2 activator, inhibited the degradation of IkBα thereby reduced NfκB activity (104). In addition, a significant reduction in ICAM-1, Monocyte Chemo attractant Protein-1 (MCP-1), and TGFβ-1 expression were also reported in diabetic nephropathy mice exposed to curcumin (Figure 5) (104). In the aorta of diabetic mice, sulforaphane treatment restored aortic levels of Nrf2 and Nrf2-dependent antioxidant gene expression and prevented the DM–induced increases in wall thickness, fibrosis, inflammation (tumor necrosis factor-α and vascular cell adhesion molecule-1 expression) and apoptosis (103). Similarly, in STZ-induced diabetic mice suffering from neuropathy, increasing the expression of Nrf2 through pharmacological agents decreased the NfxB and proinflammatory cytokines TNF-α and IL-6, iNOS and COX-2 in the sciatic nerves (105). In conclusion, activation of Nrf2 using pharmacological agents helps in reducing the complications of diabetes.

(IV) Role of Nrf2 in glucose metabolism and insulin resistance
In addition to regulating antioxidant genes, recent studies have ascribed a critical role for Nrf2 in the regulation of metabolism of carbohydrates, proteins, and lipids (106). For instance, Nrf2 regulates glucose metabolism by upregulating Slc2a1, Hk2 and Pkm2 expression (107). In addition, Nrf2 down-regulated the expression of G6PC, cAMP-CREB pathways (28). Therefore, Nrf2 has a direct role in regulating the expression of genes coding for proteins that have a role in the metabolism of glucose in such a way that promotes the utilization while suppressing the glucose production in peripheral tissues (Figure 5). Nrf2 also decreases the expression of gluconeogenesis-related genes, including fructose-1,6-bisphosphatase 1, peroxisome proliferator-activated receptor coactivator 1α (PGC1α or PPARGC1α) and Nr4a2, in db/db mouse liver (28). In line, it has been shown that Nrf2 redirects glucose into the anabolic Pentose Phosphate Pathway under the sustained activation of PI3K-Akt signaling (108).

While many studies have demonstrated a beneficial effect of activating Nrf2 in controlling the metabolism of glucose, recent studies, in contrary, have claimed a positive effect on insulin sensitivity only when Nrf2 levels were reduced (109). For example, Nrf2 knock out mice fed high-fat diet (HFD) for 180 days showed more glucose-tolerance and insulin-sensitivity than wild-type mice (109). In Lep (ob/ob) and Nrf2 KO mice, glucose and insulin intolerance is worsened compared with the Lep(ob/ob) mice, suggesting Nrf2 regulates genes involved in the glucose uptake and tolerance thus considered to be involved in impairing the insulin resistance (110). In addition, Nrf2 KO mice showed transient insulin/IGF-1 resistance in primary hepatocytes (111).

Depletion of Nrf2 using siRNA in the hepatic HepG2 cell line impaired AKT phosphorylation, which subsequently increased GSK3 activity and insulin resistance (112). Increased GSK-3 activity, in turn, reduces the nuclear accumulation of Nrf2 by translocating Nrf2 from the nucleus (113). For example, genetic ablation of GSK-3β using siRNA restored nuclear accumulation of Nrf2, suggesting that inhibition of GSK-3β can improve Nrf2 function in diabetes (Figure 3). Therefore, in conclusion, inhibition of GSK-3 not only improves insulin sensitivity but also enhances Nrf2, hence can be considered as a potential target for treating diabetic complications (113).
Figure 5. Schematic representation demonstrating various signaling cascades modulated through the Nrf2 pathway in diabetes: Nrf2-activators promote the nuclear translocation of Nrf2 thereby reduce diabetes by upregulating glycolytic enzymes hexokinase (HK), phosphofructokinase-1 (PFK1) and glyceraldehyde 3-phosphate dehydrogenase (G3PD), and enzymes of hexose monophosphate shunt pathway in particular glucose-6-phosphate dehydrogenase (G6PD). In addition, Nrf2 inhibits the enzymes of gluconeogenesis G6-phosphatase and Fructose1,6-bis phosphatase. Furthermore, Nrf2 target genes protect pancreatic β-cells from ROS-induced damage by upregulating NQO1 and SOD levels. Additionally, Nrf2 controls cell proliferation, apoptosis, autophagy and angiogenesis in diabetes (24).

b. Nrf2 protects pancreatic β-cells from oxidative stress-induced cellular damage
Pancreatic β-cells are more susceptible to oxidative stress, compared to other cell types, due to their low antioxidant capacity (114). For example, MIN6 cells, a representative pancreatic β-cell line, and islets isolated from Nrf2-knockout mice were more vulnerable to H₂O₂ induced cytotoxicity and apoptosis (115). In addition, Nrf2 null cells expressed reduced levels of antioxidant enzymes such as γ-glutamylcysteine synthetase and glutathione (116). However, pretreatment of MIN6 β-cells with Nrf2 activators protected the cells from high levels of H₂O₂-induced cell damage (115). Mechanistically, activation of Nrf2 inhibited the phosphorylation of JNK by inducing glutamate-cysteine ligase (GCL) expression, and the production of GSH, thus inhibiting the β-cell apoptosis (16). In the same way, in INS cells, the Nrf2 activator, Lithospermate B protected cytokines induced apoptosis by alleviating the phosphorylations of p38 and JNK via Nrf2-HO1 and Sirt1 signaling cascades (117, 118). Hence, activation of Nrf2 is a viable strategy to inhibit inflammatory responses.

c. Nrf2 activators as anti-diabetic agents
Based on several preclinical and cell-based studies it is now clear that activation of Nrf2 helps in providing protection to pancreatic beta cells thereby reduce diabetes complications (119). Therefore, targeted activation of Nrf2 using pharmacological agents
is considered as one of the ways to treat diabetes (48). Screening of phytochemicals for the ability to activate Nrf2 identified sulfur containing compounds, flavonoids and phenolic compounds as potential candidates for further studies. Mechanistically, these activators promote the expression of Nrf2 by (i) interacting with critical cysteine residues of Keap1; (ii) acting on upstream kinases such as Akt, ERK, PI3K, PKC and JNK causing the release of Nrf2 from Keap1. (iii) inhibiting ubiquitin-mediated proteosomal degradation of Nrf2 thereby prolong its half-life, (iv) interacting with critical cysteine residues of Nrf2 causing its release from Keap1 (Figure 6).

Some of the known Nrf2 activators that interact with Keap1 cysteine residues include Sulforaphane (SFN), Cinnamic aldehyde (CA), Tert-Butylhydroquinone (tBHQ), Curcumin (CUR), and Bardox-olone methyl (48). Other activators Epigallocatechin gallate (EGCG), Resveratrol (RSV), Cinnamic aldehyde (CA), Magnesium lithospermate B (MLB) and tBHQ exhibit Nrf2 activation potential by promoting upstream kinases (120). Ubiquitin inhibitor MG132 stabilizes Nrf2 by preventing its degradation in cells (121).

**Figure 6. Possible mechanisms by which Nrf2 activators promote Nrf2/Keap1 pathway:** Sulforaphane, Curcumin, Cinnamic Aldehyde, Bardoxolone methyl and tert-Butylhydroquinone, activate Nrf2 signaling by interacting with critical cysteine thiol residues of Keap1 (48). On the other hand, Resveratrol, Cinnamic Aldehyde, Magnesium lithospermate B, Epigallocatechin gallate, tert-Butylhydroquinone, act on upstream kinases such as Akt, ERK, PI3K, PKC and JNK causing the phosphorylation of Nrf2 and its translocation to the nucleus (120). MG132, an inhibitor of ubiquitination, act at the level of proteasome and inhibit proteosomal degradation of Nrf2 (121).

(I) Sulforaphane (SFN)

Sulforaphane is an organosulfur compound obtained from cruciferous vegetables such as broccoli, brussels sprouts or cabbages and has been reported to prevent cancer, inflammation, and diabetes (50). Mechanistically, sulforaphane modifies specific cysteine (C151, C489, and C583) residues that act as "sensors" of oxidative stress in Keap1 thereby prevent its interaction with Nrf2 (Figure 6) (122). Several studies have shown that sulforaphane protects diabetes-induced micro and macrovascular damage by triggering
the expression of several antioxidant enzymes via Nrf2 pathway (123). A study by Wenpeng et al. (2012) (124) using an STZ-induced diabetic mouse model revealed that sulforaphane treatment positively attenuated high glucose-induced mesangial cell hypertrophy by Nrf2-mediated TGFB signaling repression. Additionally, oral or subcutaneous administration of sulforaphane diminished oxidative stress generation, TGF-β1 expression, fibronectin and type IV collagen deposition in the kidney of STZ-induced diabetic rats/mice (125, 126). Intrapitoneal injection of sulforaphane at 5mg/kg ameliorated experimental diabetic nephropathy by suppressing glycogen synthase kinase 3β/Fyn-signaling cascade through Nrf2 (127). In rat aorta and HUVEC cells, sulforaphane treatment markedly inhibited the AGEs-induced increase in RAGE, monocyte chemo attractant protein-1 (MCP-1), ICAM-1 and VCAM-1 gene expression (128). In rat insulinoma cells, sulforaphane increased the accumulation of Nrf2 protein in the nucleus and decreased cell death induced by inflammatory cytokines implying sulforaphane may block the β-cell apoptosis or its dysfunction caused by increased oxidative stress (129). In addition, several studies have validated the protective role of sulforaphane against diabetes-induced endothelial, cardiac and aortic damage as well as testicular cell death mediated by the upregulation of Nrf2 (103, 130).

(II) Curcumin

Curcumin (Diferuloylmethane) is a hydrophobic, low molecular weight flavonoid present in the rhizome of Curcuma longa. Curcumin is a potent antioxidant with good anti-inflammatory activities and is reported to be useful for treating diseases associated with oxidative stress and inflammation (131). A number of scientific reports demonstrated the protective effect of curcumin against oxidative stress mediated cardiomyopathy (132), neuropathy (133), testicular damage (134) and liver dysfunction (135). In high fructose-fed male Wistar rats, curcumin decreased the serine phosphorylation of IRS-1 and increased tyrosine phosphorylation of IRS-1 thereby prevented insulin resistance (136). Curcumin also regulates SREBPs target genes and metabolism associated genes in the liver or adipose tissues, which may directly contribute to the lower lipid level and improvement of insulin resistance in HFD induced obese mice (137). Additionally, curcumin reduced high glucose-induced cardiomyocyte apoptosis by inhibiting NADPH-mediated oxidative stress and prevented insulin resistance by increasing the activity of PI3K/Akt (138, 139). In a study by Vivian et al. (2013) (140) normalization of renal dysfunction and lipid peroxidation in STZ rats was reported to be associated with inhibition of PKC-α and PKC-β1 by curcumin. Also, curcumin administration in STZ diabetic animals markedly decreased the infiltration of renal macrophages, plasminogen activator inhibitor-1 (PAI-1), fibronectin expression and proinflammatory cytokines such as TNF-α and IL-1β along with NFKB inhibition (104, 141, 142). In pancreatic tissue of STZ induced cellular damage, curcumin decreased the levels of proinflammatory cytokines TNF-α, IL1-β and IFN-γ, increased the level of cellular defense proteins Nrf-2 and HO-1, and glucose transporter (GLUT-2) (143-145). Further, an increase in the number of small pancreatic islets and decreased lymphocyte infiltration in pancreatic islets was also reported with curcumin administration (146). Also curcumin has been reported to show its cellular effects via various other molecular mechanisms like (1) inhibition of the JNK pathway through suppression of AP-1, Phospho-p38 mitogen-activated protein kinase, NF-kB signaling, (2) modulation of transcription factors like PPAR-γ expression, enzymes (e.g., COX2, 5-LOX, iNOS, and hemeoxygenase-1), cell cycle proteins (e.g., cyclin D1 and p21), and (3) Wnt signaling (147-149).

(III) Resveratrol (RSV)

Resveratrol is a polyphenolic stilbene derivative (3, 5, 4-trihydroxy-trans-stilbene) found in grapes, peanuts, blueberries, rheum, bulb flower, mulberries, and cranberries, red wine. The resveratrol has received considerable attention due to its accessibility to the general population and a myriad of potential health benefits (150, 151). It is considered as a
potential Nrf2 activator and a successful small molecule in treating diabetes, both in preclinical and clinical studies (152). Several lines of evidence suggest that resveratrol acts through multiple pathways, including AMP-activated protein kinase (AMPK), sirtuin 1 (SIRT1) and nuclear factor erythroid 2-related factor 2 (Nrf2) to protect against cell dysfunction and improve insulin sensitivity in various diabetic models. In the liver of diabetic rats, resveratrol promoted a reduction in Slc2a2/ GLUT2, Pck1, and G6pc mRNAs expressions and increased the hepatic glycogen content, and SIRT1 protein (153). Resveratrol also decreases the activities of key enzymes of gluconeogenesis such as phosphoenol pyruvate carboxykinase (154) and increases the activity of hexokinase and pyruvate kinase (155). Taken together these enzymatic changes lead to the shifting of the metabolic pathways toward reduced hepatic glucose output and show the ability of resveratrol in glucose metabolism and maintaining the insulin action (156). In cardiomyocytes and HUVEC cells resveratrol protected against high glucose-induced apoptosis through suppression of NADPH oxidase-derived ROS generation and maintained endogenous antioxidant defenses mainly mediated by AMPK pathway (157, 158). In type 2 diabetic patients, administration of 10mg/d resveratrol improved insulin sensitivity via the protein kinase B signaling (159). In high-fat diet induced renal injury, resveratrol attenuated structural disorders and renal dysfunction by increasing lipolysis through the activation of the PPAR-α signaling pathway. Besides, resveratrol is also shown to exert its protective role against diabetic complications by enhancing the antioxidant status through Nrf2 and decreases the inflammatory responses such as p38 MAPK, NfkB, JNK, TNF-α, IL-1β, IL-6 (160-164).

(IV) MG132

MG132 (Carbobenzoxyl-L-leucyl-L-leucyl-L-leucinal) is a naturally occurring, potent, reversible, and cell-permeable triterpene proteasome inhibitor derived from a Chinese medicinal plant. MG132 reduces the breakdown of Nrf2 by inhibiting ubiquitin-mediated proteasomal degradation pathway (Figure 6). As a result, MG132 stimulates the translocation of Nrf2 into the nucleus to promote a set of antioxidant cytoprotective genes (165). Primarily, Luo et al. (2011) (166) evidenced that MG132 at 10µg/kg daily for 3 months could mitigate the symptoms of STZ induced-diabetes in rats. Rats treated with MG132 showed renal protection by decreasing proteinuria, basement membrane thickening and glomerular mesangial expansion. MG132 also reduced kidney markers of oxidative stress and increased protein levels of Nrf2 and its downstream antioxidant enzymes. In transgenic type 1 diabetic (OVE26) mice with renal dysfunction, MG132 administration improved the renal structural and functional alterations associated with increased Nrf2 expression and transcriptional upregulation of Nrf2-regulated antioxidants (167). Recently, it has been shown that MG132 attenuate diabetes in rat glomerular mesangial cells by inhibiting Smad7 ubiquitin, SnoN degradation and TGF-β activation (168, 169). Likewise, an in vitro study showed protection of neonatal rat cardiomyocytes against H₂O₂ mediated oxidative stress when treated with 0.5µM MG132 for 48h. Mechanistically, MG132 reduces the levels of intracellular ROS by upregulating SOD, HO-1, and CAT expression (170). In addition, MG132 could also down-modulate NfkB to mitigate inflammatory responses (121).

(V) Tert-butylhydroquinone (tBHQ)

Tert-butylhydroquinone is a synthetic phenolic antioxidant, which exerts its antioxidant function via a Keap1/Nrf2 pathway (171). tBHQ is currently approved for human use by both the World Health Organization and the Food and Agriculture Organization (172). Several scientific reports suggest that tBHQ treatment elicits cytoprotective actions in different organs under various pathological conditions. For example, systemic or local intra-cerebroventricular treatment with tBHQ in an ischemic stroke model in rats significantly reduced the infarct size and neurological deficits (173). Likewise, there is
evidence showing that administration of tBHQ in rats suppressed renal damage and oxidative stress after ischemia and reperfusion injury (174). Pretreatment with tBHQ potently inhibited (1) lipopolysaccharide-induced microglial activation, inflammation, and apoptosis (175), (2) hydroxydopamine-induced oxidative injuries (176) and (3) hypochlorous acid-induced macrophages damages (114). tBHQ also maintained the testicular integrity and activated the Nrf2 signaling pathway in scrotal heated mice (177, 178). In a continuity study by the same author, in scrotal heat-induced mice, tBHQ treatment markedly increased Nrf2 protein expression in cytoplasm and nuclei of interstitial cells, accompanying with an elevated mRNAs expression of Nrf2 and Nrf2-regulated genes in mice testes (179). In mice with type 1 diabetes, chronic treatment with tBHQ reduced the degree of glomerular fibrosis and ameliorated proteinuria (180). In addition, pretreatment of MIN6 β-cells with tBHQ in combinations with CDDO-Im and dimethyl fumarate (DMF), protected the cells from high levels of H₂O₂-induced cell damage by persistent activation of Nrf2 (115). Very recently, tBHQ have been shown to confer protection to islets and retina of type 2 diabetic rats by the secretion of insulin in diabetic rats, lowering glucose levels, suppressing the expression of VEGF and inducing the expression of HO-1 (181).

(VI) Bardoxolone methyl (BARD)

The synthetic triterpenoid bardoxolone methyl is the most potent known activators of the Nrf2 pathway (182). BARD modulates oxidative stress and inflammation by suppressing inflammatory signaling pathway and proinflammatory cytokines while activating anti-inflammatory antioxidant signaling pathways (183–185). Because of this dual role human and animal studies have illustrated anti-obesity, anti-diabetic, and anti-inflammatory actions of BARD. In a previous study, it has been reported that BARD is effective in preventing obesity-associated complications such as cognition and inflammation in the brain and peripheral tissue of HFD-fed mice (186). In white fat, particularly, oral BARD prevents fat deposition and invasion of macrophages, inflammatory cytokines, and stress-activated proteins (186, 187). In addition, BARD ameliorates aristolochic acid (AA)-induced acute kidney injury by increasing the renal expression of Nrf2, HO-1, and NQO1 (188). In a recent study, 2-week administration of BARD decreased hepatic inflammation in diet-induced obese mice (189). In a similar way, oral administration of the derivative of BARD 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDOIm) prevents HF diet-induced obesity and attenuates diabetes in mice (28, 190). BARD could also prevent HFD induced insulin resistance by modulating the (1) level of protein such as tyrosine phosphatase 1B, FOXO1 and (2) expression of the insulin receptor (IR), IRS-1 and glucose-6-phosphatase (G6Pase) genes (191). In the same study, BARD also prevented fat accumulation in the liver and decreased the expression of β-oxidation gene peroxisomal acyl-coenzyme A oxidase 1 (ACOX) and STAT3 protein levels.

(VII) Cinnamaldehyde (CA)

Cinnamaldehyde is a chief substance found in the essential oil from both the bark and leaves of cinnamon varieties (192). Several lines of evidence suggest antioxidant, antiangiogenic, anti-inflammatory and anti-diabetic activities of cinnamon that were attributed to the cellular effects of CA (193–195). CA protected against ROS produced under hyperglycemic conditions and thus protected pancreatic β-cells and exhibited anti-diabetic property (196). It also prevented the development of hypertension during insulin deficiency and insulin resistance by regulating vascular contractility and exerting an insulinovertopic effect (196). Administration of CA showed potent hypoglycemic and hypolipidemic effect by lowering blood glucose, total cholesterol, and triglycerides and increasing the HDL cholesterol in STZ induced diabetic rats (197). Additionally, in STZ induced diabetes model, CA had a Nrf2 dependent effect on the antioxidants status of the rat kidney (125). Wondrak et al. (2010) (198) showed that CA treatment in human colon
cancer cells and non-immortalized primary fetal colon cells upregulated the cellular protein levels of Nrf2 and Nrf2 target genes. Furthermore, the phosphorylation of ERKs/JNK/p38 MAPK induced by high glucose was also inhibited by CA (199). CA also inhibit high glucose-induced hypertrophy by decreasing cell size, cellular hypertrophy index, protein levels of collagen IV, fibronectin, and α-smooth muscle actin in renal tubular epithelial cells (200). Moreover, CA inhibited the AGEs induced JAK2-STAT1/STAT3 activation, RAGE/p27/collagen IV protein levels, and cellular hypertrophy in human renal proximal tubular cells (201).

(VIII) Lithospermate B (LB)

Magnesium lithospermate B is also known as “Danesh” is the active component in the water-soluble fraction of Salviae miltiorrhizae, which has been used in China for hundreds of years to treat numerous ailments (202). LB showed its potent antioxidant beneficial characteristics by free-radical scavenging, inhibition of lipid peroxidation (203, 204) and activation of the Nrf2-ARE-NQO1 transcriptional pathway (205, 206). On the other hand, LB inhibits the enzyme aldose reductase, which is a key component of the polyol pathway involved in the pathogenesis of diabetic complications (207). LB had an anti-apoptotic effect on cytokines induced apoptosis in INS cells in vitro and a preventive effect on the development of type 2 diabetes in an Otsuka Long-Evans Tokushima Fatty (OLEFT) animal model in vivo (117). In this study, the anti-apoptotic effects of LB were evidenced by the down-regulation of the apoptotic pathways of the c-Jun NH2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), and the up-regulation of the anti-apoptotic pathway of Nrf2/HO-1 and Sirt1 expression (117). Furthermore, LB inhibits other major molecular pathways related to diabetic vascular complications, which include the hyperglycemia-induced hexosamine pathway, PKC activation and transforming growth factor TGF-β (205). In hyperglycemia-induced endothelial cells, LB rescued the inhibition of endothelial nitric oxide synthase (eNOS) activity, eNOS phosphorylation and increased Nrf-2 activation in a phosphoinositide 3-kinase/Akt pathway-dependent manner (208). In high-fat-diet-induced metabolic syndrome supplementation of LB improved obesity, hyperlipidemia, hyperglycemia, glucose intolerance, insulin resistance, hepatic steatosis through downregulating CD36, sterol regulatory element-binding transcription factor 1c (SREBP1c) expression and up-regulating lipolytic transcription factor peroxisome proliferator-activated receptor-α (PPARα) expression (209). Table 2 listed the Nrf2 activators reported in the treatment of diabetes.
Table 2. List of Nrf2 activators evaluated in *in vitro* and *in vivo* models

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Name of Nrf2 Activators</th>
<th>Experimental Model Tested</th>
<th>Diabetes Condition</th>
<th>Mechanism(s) and References</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sulforaphane (SFN)</td>
<td>Eight-week-old WT C57BL/6J (Nrf2[−/−]) and 129S1 (MT[−/−]) male mice Induction of Type 2 diabetes by feeding HFD SFN at 0.5 mg/kg for 4 months with HFD</td>
<td>Diabetic nephropathy</td>
<td>Sulforaphane enhanced renal Nrf2 expression (210).</td>
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<td>C57BL/6J male HFD-fed for 3 months and a single dose of STZ 100 mg/kg i.p. SFN 0.5 mg/kg for 5 days each week for 4 months, subcutaneously</td>
<td>Cardiomyopathy</td>
<td>Down-regulated diabetes-induced PAI-1, TNF-α, CTGF, TGF-β, 3-NT, and 4-HNE expression Inhibited LKB1/AMPK pathway Increased Nrf2, and target genes HO-1 and NQO-1 (211).</td>
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<tr>
<td>2.</td>
<td>Resveratrol (RSV)</td>
<td>Four-week-old male Balb/C mice Methylglyoxal 1% in water, daily for 12 weeks orally Resveratrol 10 mg/kg daily for 12 weeks orally</td>
<td>Pancreatic Damage</td>
<td>Promoted Nrf2-phosphorylation (212).</td>
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<td>Six-week-old male Wistar rats Single dose of STZ 50 mg/kg i.p. after administration of nicotinamide 110 mg/kg i.p. Resveratrol 5 mg/kg for 30 days orally</td>
<td>Diabetic nephropathy</td>
<td>Increased the expression of Nrf2 and target genes γ-GCL, μ-GST and HO-1 in renal tissues (213).</td>
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<td></td>
<td></td>
<td>HepG2 Cells with MG (500 μM) and Resveratrol (50 μM) for 24h</td>
<td>Insulin Resistance</td>
<td>Enhanced Nrf2 nuclear translocation and its activation via ERK phosphorylation. Promoted Nrf2 target gene HO-1 levels (98).</td>
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<tr>
<td>3.</td>
<td>MG-132</td>
<td>Female OVE26 mice MG-132 -10μg/kg daily for 3 months, i.p</td>
<td>Diabetic Cardiomyopathy</td>
<td>Upregulation of Nrf2 expression and downregulation of NF-κB (121).</td>
</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Treatment Details</td>
<td>Effects</td>
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<td>4.</td>
<td>Oltipraz</td>
<td>Male C57BL/6J mice fed with HFD (45% energy from fat) Oltipraz -0.75 g/kg diet, For 28 weeks</td>
<td>Insulin resistance and obesity Increase nuclear translocation of Nrf2 (112).</td>
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<td>5.</td>
<td>Curcumin</td>
<td>H9C2 cells treated with palmitate 500 μM and curcumin (20 μM) for 1h Eight –week-old Male C57BL/6 mice fed HFD for 8 weeks Curcumin - 50 mg/kg daily for 8 weeks, orally</td>
<td>Heart Increased the expression of Nrf2, but inhibited NF-kB (214).</td>
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<td>6.</td>
<td>Quercetin</td>
<td>High-carbohydrate, high-fat-diet-fed Male Wistar Rats Quercetin - 0.8 g/kg for 8 weeks with diet</td>
<td>Cardiovascular and Hepatic complications Increased the expression of Nrf2, HO-1, and CPT1, but lowered the levels of NF-kB (215).</td>
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<td>7.</td>
<td>tetra-Butylhydroquinone (tBHQ)</td>
<td>(Nrf2+/+) and (Nrf2−/−) CD1/ICR mice tBHQ (25 mg/kg) for 3 consecutive days, and DOX (20 mg/kg) once on day 2 i.p</td>
<td>Cardiotoxicity Enhanced expression of Nrf2 and its downstream antioxidant genes, HO-1 and NQO-1(216)</td>
<td></td>
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<td>8.</td>
<td>Pentaerithritol Tetranitrate (PETN)</td>
<td>Male Wistar rat STZ 60 mg/kg, i.p. PETN (15 mg/kg/day) or ISMN; (75 mg/kg/day ) for 7 weeks, p.o.</td>
<td>Vascular dysfunction Increased expression of Nrf2 and HO-1(217).</td>
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<td>9.</td>
<td>Dihydro-CDDO-trifluoroethyl amide (Dh404)</td>
<td>Male C57Bl/J6 and ApoE2/2 mice STZ 100 mg/kg/day on 2 consecutive days 10 or 20 mg/kg dh404, daily, 5 weeks, oral gavage</td>
<td>Atherosclerosis, Diabetic Kidney Upregulation of Nrf2 responsive genes, HO1, NAD(P)H-quinone oxidoreductase 1 and GST with inhibition of profibrotic fibronectin, collagen I, and proinflammatory interleukin-6 (218).</td>
<td></td>
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<tr>
<td>10.</td>
<td>Cinnamic aldehyde (CA)</td>
<td>Nrf2+/+ and Nrf2−/− C57BL/6 mice Single dose of STZ -50 mg/kg, i.p. CA 25/50 mg/kg for 16 weeks, daily orally</td>
<td>Diabetic Nephropathy Nrf2 activation suppressed the expression of TGF-b1, ECM, and p21(125).</td>
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11. Telmisartan

Male C57BL/6-Akita diabetic and C57BL/6-wild-type (C57BL/6-WT) non-diabetic mice

Telmisartan - 5mg/kg/day orally or amlodipine - 5mg/kg/day for 4 weeks.

Diabetic nephropathy

Upregulation of renal SOD enzyme suppression of NAD(P)H oxidase and upregulation of Nrf2 (219).

12. Epigallocatechin-3-gallate (EGCG)

Male Wistar rats with single dose

Cisplatin - 7 mg/kg i.p.

EGCG 100 mg/kg for 12 days p.o.

Nephrotoxicity

Increased the levels of Nrf-2 and HO-1, but inhibited NF-κB and HNE (220).

13. Diallyl sulfide (DAS)

Wistar male albino rats

Gentamicin (100 mg/kg) for six consecutive days i.p.

DAS (150 mg/kg) for 6 days, i.p.

Nephrotoxicity

Activation of Nrf2 and the suppression of iNOS, TNF-α and NF-κB (221).

14. Naringenin (NAR)

H9C2 cells with H2O2 stress of 150 μM for 1 h

Treated with 50μM of naringenin for 24h

Cardiomyoblast

Upregulated Nrf2 and its target genes, Upregulated Akt and downregulated NF-κB and caspase3 genes (222).

Male Sprague-Dawley rats with CCl4 10 ml/kg for 5 days i.p.

Naringenin (50 mg/kg) for 7 days orally

Hepatocytes

Increased Nrf2 and HO-1 expression

Down-regulated TNF-α, iNOS, COX-2 (223).

15. Pterostilbene (PTS)

INS 1-E cell

PTS (0–16μM) treatment up to 48h, followed by STZ (10mM) for 1 h

Pancreatic β-cell damage

Upregulation of Nrf2, HO-1, SOD, CAT, GPX, and Bcl-2,

Down regulation of Bax and caspase-3 expression (58).

d. Knockout studies demonstrating the role of Nrf2 in the development of diabetes

Gene knockout experiments are one of the best explored proof-of-principle studies widely used to study the role of a particular gene (224). In order to demonstrate the role of Nrf2 in the development of diabetes, several gene-knock out and knock-in experiments have been carried out by several investigators and the results documented (225). In general, Nrf2 counteracts not only high-glucose-induced oxidative stress and but also reduce downstream inflammatory responses in diabetes (226). For example, a study has shown that elevated oxidative stress markers such as oxidized low-density lipoprotein (Ox-LDL), 4-hydroxynonenal (HNE) and transforming growth factor-β that are observed in diabetic animal models, have been shown to activate Nrf2 (227). Likewise, in the early onset of diabetes, a significant increase in the levels of Nrf2 and HO-1 were observed, indicating that these proteins provide an adaptive response to counteract oxidative stress diabetes (94). However, at the later stage of diabetes, a decline in the activity of Nrf2 was reported (228).
Effect of knocking down Nrf2 in diabetic cardiomyopathy: The role of Nrf2 in diabetic cardiomyopathy was investigated in vivo by using the Nrf2 knockout (KO) mice and streptozotocin (STZ)-induced diabetic model (225). Nrf2 KO mice were highly sensitive to STZ-induced diabetic lesions to develop severe cardiomyopathy compared to wild-type (WT) control, suggesting that loss of Nrf2 synergizes with high glucose to induce an elevated oxidative condition in the heart of Nrf2 KO mice resulting in severe myocardial lesions (229). In addition, neonatal and adult cardiomyocytes isolated from Nrf2−/− mice exhibited higher ROS under basal conditions compared with WT controls followed by an increase in cell apoptosis (229). Furthermore, an increase in the oxidative damage to DNA was observed in the diabetic Nrf2−/− heart (229).

The role of knocking down Nrf2 in diabetic nephropathy: STZ-treated Nrf2−/− mice developed renal impairment much prior to wild-type (WT) mice, with a significant decline in creatinine clearance rate as early as 6 weeks compared to 10 weeks for WT (94). The urinary excretion of nitric oxide metabolites and the occurrence of 8-nitroguanosine were also increased in Nrf2−/− mice after STZ induction evidencing hyperglycemia increased oxidative and nitrosative stress to a greater extent and accelerated early stage renal injury in Nrf2 KO mice as compared to WT mice (94). Moreover, diabetic Nrf2−/− mice revealed increased expression of the fibronectin, proinflammatory cytokines, transforming growth factor-β (TGF-β), compared to diabetic WT mice (94).

In addition to cardiomyopathy and nephropathy, knockdown of Nrf2 results in the development of diabetic retinopathy and neuropathy (100). Diabetic mice lacking Nrf2 showed a significant increase in the superoxide levels, reduction in retinal glutathione along with an increase in TNF-α protein compared to wild-type mice harboring functionally active Nrf2 (100). Nrf2 null mice exhibited early onset of blood-retina barrier dysfunction and exacerbation of neuronal dysfunction in diabetes.

e. Nrf2 activators in clinical trials

Since Nrf2 is a key antioxidant regulator in cells, many researchers have made attempts to identify potent Nrf2 activators from natural sources (50). However, only a few activators could reach the clinical trials (230). Although Nrf2 activators such as Sulforaphane, Resveratrol, Curcumin, are known to suppress the complications of diabetes in experimental animal models, their utility in a clinical setting for treating ROS-induced diseases such as diabetes is limited (230). Among different Nrf2 activators sulforaphane was much studied, hence, a brief account of the use of sulforaphane for treating diabetes is discussed. A clinical study reported that sulforaphane administration for 4 weeks in type 2 diabetes patients decreased serum MDA and Ox-LDL concentration while increasing total antioxidant capacity that might be useful to improve antioxidant status in such patients (231). In addition, sulforaphane markedly decreased serum insulin, TG, LDL, and increased the level of HDL in diabetic patients (232, 233). Sulforaphane also minimized the effect of inflammatory cytokines such as TNF-α and IL-6 (234).

Another Nrf2 activator explored clinically is curcumin (235, 236). Curcumin treatment in type 2 diabetic patients significantly improved endothelial function and reduced the oxidative stress and inflammatory responses such as IL-6, TNFα, and endothelin1 (235). In addition, curcumin ameliorated proteinuria, transforming growth factor β and IL-8 in diabetic nephropathy patients (236). Recently a report analyzed the effect of the curcumin on the redox status and Nrf2 activation in the Mexican patients with nondiabetic and diabetic proteinuric chronic kidney diseases (CKD) (85). Briefly, patients with nondiabetic proteinuric CKD and diabetic proteinuric CKD were intervened with placebo or curcumin 320mg/day for 8 weeks and levels of CKD compared before and after treatment (85).
plasma, curcumin attenuated lipid peroxidation and enhanced the antioxidant capacity in both nondiabetic and diabetic proteinuric CKD subjects (85). Surprisingly, no effect of curcumin was observed in the antioxidant enzyme activities or Nrf2 activation (85). Also, curcumin did not improve proteinuria, glomerular filtration rate, or lipid profile (85). In contrast to these results, supplementation of curcumin at the dose of 500mg/day for a period of 15-30 days in T2DM patients activated the Nrf2 system and its downstream antioxidant enzymes in blood lymphocytes (237). Supplementation of curcuminoids to T2DM patients significantly decreased fasting blood glucose, HbA1c, TG and insulin resistance index (HOMA-IR) (237). In another study, diabetic subjects were randomly assigned to receive either curcumin or placebo capsules, and changes in β cell functions (homeostasis model assessment [HOMA]-β, C-peptide), insulin resistance (HOMA-IR) and anti-inflammatory cytokine levels were monitored (238). After 9 months of treatment, the subjects of the curcumin-treated group showed a better overall function of β-cells with higher HOMA-β, lower C-peptide levels and exhibited a lower level of HOMA-IR when compared with the placebo group (238).

Resveratrol is one of the most studied Nrf2 activator in clinical trials (152). Supplementation of trans-resveratrol (5mg) daily for 4 weeks significantly improved insulin sensitivity (HOMA-IR), lowered blood glucose levels in type 2 diabetic men compared with placebo (159). In addition, resveratrol treatment has shown an increase the activity of Akt, which ultimately promotes the uptake of glucose and activate glycogenesis (159). However, more recently, few studies have shown conflicting results (239). For instance, in a double-blind, randomized, placebo-controlled trial, 192 T2DM patients were randomized to receive resveratrol 500mg/day, resveratrol 40mg/day or placebo for 6 months (239). No significant differences in the weight, fasting glucose, glycated hemoglobin, insulin, free fatty acids, liver transaminases, interleukin-6 were observed in the resveratrol supplemented group compared to placebo (239). Supporting this observation, a separate study testing the administration of resveratrol, at a dose of 500mg twice daily for 5 weeks reported no significant effect on body weight, glucagon-like peptide 1 (GLP-1), glycemic control and glycated haemoglobin in T2DM patients (240).

The benefits of Nrf2 activators in diabetes are particularly attractive, owing to the public health burden of this disease and of its chronic complications. Many human trials reported improvements in glycemic control, insulin sensitivity and other metabolic parameters after Nrf2 activators supplementation (241, 242). However, few clinical studies have shown conflicting results, which may be due to differences in the dosage and the duration of supplementation, the bioavailability of compounds, and the characteristics of the patients studied (243, 244). Hence, further studies are required to explore the exact role of Nrf2 activators in clinical trials.
f. Naringenin

One among the naturally occurring Nrf2 activators is naringenin (223). It is a bioflavonoid, well absorbed in the gastro-intestinal tract and its glucuronide conjugates predominate in the plasma and other tissues up to 18h after oral administration (245). Plasma levels of naringenin and its metabolites are reported to reach 5μM at 2h post-gavage of 50mg/kg b.w. of naringenin, which is similar to plasma concentrations of naringenin in healthy human volunteers following ingestion of grapefruit juice (246). Naringenin has been pharmacologically evaluated for antioxidant (247), anti-inflammatory (248), anticancerous (249), anti-atherosclerotic (68), hepatoprotective (250), nephroprotective (251) and immuno-modulatory (252) activities.

Prior studies have demonstrated that naringenin treatment significantly protected against ethanol-induced hepatotoxicity, cisplatin-induced nephrotoxicity, and inhibited phosphorylation of nuclear factor kappaB (NFκB) subunit p65 and mitogen-activated protein kinases (MAPK) in daunorubicin-induced nephrotoxicity (251, 253). In addition, naringenin reduced the inflammation by targeting cyclooxygenase (COX)-2 in ethanol-induced liver injury (254). In vitro studies have shown that naringenin modulates hepatic very-low-density lipoprotein (VLDL) apoB100 production through activation of PI3K and MAPK/ERK proteins (255).

Recent studies have demonstrated that naringenin could activate Nrf2 (223). Esmaeilli et al. (2014) reported that naringenin suppressed the hepatic inflammation in CCL4 treated rats via the activation of the Nrf2 pathway (223). Likewise, naringenin provided protection against 6-hydroxy dopamine-induced oxidative stress in SH-SY5Y cells and in vivo via Nrf2 (256). However, not much is known about whether naringenin reduces oxidative stress in MIN6 pancreatic β-cells through Nrf2 signaling. Therefore, in the current study, the efficacy of naringenin for activating Nrf2 in pancreatic beta cells line MIN6 was measured using Nrf2−Keap1 reporter complementation system (257). Next, the ability of naringenin to protect MIN6 cells from streptozotocin (STZ)-induced damage was measured. In addition, the anti-diabetic potential of naringenin in STZ-induced diabetic mice was assessed.