Quercetin and its role in modulating endoplasmic reticulum stress: A review

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Abstract

The endoplasmic reticulum (ER) is the place where proteins and lipids are biosynthesized and where transmembrane proteins are folded. Both pathological and physiological situations may disturb the function of the ER, resulting in ER stress. Under stress conditions, the cells initiate a defensive procedure known as the unfolded protein response (UPR). Cases of severe stress lead to autophagy and/or the induction of cell apoptosis. Many studies implicate ER stress as a major factor contributing to many diseases. Therefore, the modulation of ER stress pathways has become an attractive therapeutic target. Quercetin is a plant-derived metabolite belonging to the flavonoids class which presents a range of beneficial effects including anti-inflammatory, cardioprotective, anti-oxidant, anti-obesity, anti-carcinogenic, anti-atherosclerotic, anti-diabetic, anti-hypercholesterolemic and anti-apoptotic activities. Quercetin also has anti-cancer activity, and can be used as an adjuvant to decrease resistance to cancer chemotherapy. Furthermore, the effect of quercetin can be increased with the help of nanotechnology. This review discusses the role of quercetin in the modulation of ER stress (and related diseases) and provides novel evidence for the beneficial use of quercetin in therapy.

Key words: Endoplasmic reticulum stress; flavonoids; quercetin.

Abbreviations:

AD: Alzheimer's disease; **AIF**: Apoptosis-inducing factor; **AST/ALT:** Aspartate/alanine transaminase; **ATF-4**: Activating transcription factor 4; **ATF-6**: Activating transcription factor-6; **Aβ**: Amyloid-β; **BCL2**: B-cell lymphoma 2; **Grp78**: Glucose-regulated protein 78; **Ca^{2+}**: Calcium; **CDK2**: Cyclin dependent kinase 2; **CHOP**: C/EBPα-homologous protein; **EDEM1**: ER degradation-enhancing α-mannosidase-like protein; **EIF2α**: Eukaryotic translation initiation factor

2 alpha; **EndoG**: Endonuclease G; **eNOS**: Endothelial nitric oxide synthase; **ER**: Endoplasmic reticulum; **ERdj4**: ER-localized DnaJ homologue 4; **ET-1**: Endothelin-1; **FDA**: Food and Drug Administration; **GADD153**: Growth arrest and DNA damage 153; **GPx**: Glutathione peroxidase; **HSP:** Heat shock proteins; **IL**: Interleukin; **iNOS**: Inducible nitric oxide synthase; **IR**: Ionizing radiation; **IRE-1**: Inositol requiring protein-1; **JNK**: c-Jun N-terminal kinase; **MDA**: Malondialdehyde; **MDR1**: Multidrug resistance mutation 1; **NLRP3**: NOD-like receptor family, pyrin domain containing-3;; **PARP**: Poly (ADP-ribose) polymerase; **PERK**: Protein kinase RNA-like ER kinase; **PI3K**: Phosphoinositide 3-kinases;; **ROS**: Reactive oxygen species; **SIRT1/AMPK**: Sirtuin1/adenosine monophosphate-activated protein kinase; **SOD**: Superoxide dismutase; **TNF-α**: Tumor necrosis factor alpha; **Tuj1**: Class III beta-tubulin; **TXNIP**: Thioredoxin-interacting protein; **XBP1**: X box-binding protein 1.

1. Introduction

Plants have long been studied for the beneficial effects of their constituents that can be used as templates for the discovery and development of new drugs (Atanasov *et al.*, 2021). Quercetin is a plant flavonoid. The latter are natural phenolic compounds with biological properties that could be exploited for the treatment of diseases such as diabetes, cancer, inflammation, cardiovascular disorders, and microbial infections. Quercetin has demonstrated anti-inflammatory, cardioprotective, anti-oxidant, anti-obesity, anti-carcinogenic, anti-atherosclerotic, anti-hypercholesterolemic, and anti-apoptotic activity. It has also been reported as an adjuvant to decrease resistance to cancer chemotherapy. Its use is recognized as safe by the Food and Drug Administration (FDA) (Salvamani *et al.*, 2014; Sultana and Anwar, 2008; Kressler *et al.*, 2011; Li *et al.*, 2020; Nathiya *et al.*, 2014; D'Andrea, 2015; Wang *et al.*, 2018). The endoplasmic reticulum (ER) is a eukaryotic and multifunctional organelle, required for protein synthesis, folding and trafficking, calcium (Ca^{2+}) storage and lipid synthesis. In this line, when the ability of the ER to fold proteins becomes saturated, ER stress occurs. ER stress refers to changes in Ca^{2+} concentrations, hypoxia-induced alterations in the cellular redox capacity, or accumulation of unfolded/misfolded proteins caused by chronic inflammation and/or viral infections (Engin and Hotamisligil, 2010). The ER stress response or unfolded protein response (UPR) is a complicated signal transduction cascade expanding from the endomembrane and activated by any disturbance in the normal metabolism of ER (Fagone and Jackowski, 2009). Importantly, ER stress has been linked to a range of health disorders including neurodegenerative and metabolic diseases, inflammation, osteoporosis, and cancer (Wang and Kaufman, 2016; Shakeri *et al.*, 2019) (**Figure 1**). Several *in-vitro* and *in-vivo* experiments have demonstrated the ability of quercetin to modulate ER stress *via* the regulation of several pathways/factors, such as the sirtuin1/adenosine

monophosphate-activated protein kinase (SIRT1/AMPK), and the signal transducer and activator of transcription (STAT)-3 (Feng *et al.*, 2019; Yang *et al.*, 2015b). In this review, we discuss the role of quercetin in the modulation of ER stress and its related diseases.

2. Endoplasmic reticulum stress

The endoplasmic reticulum (ER) is a cell organelle whose function is to synthesize and fold membrane and secretory proteins. ER is also necessary for several other cellular functions including Ca^{2+} homoeostasis, and the biosynthesis of cholesterol and phospholipids. These functions are ATP-dependent processes and require an appropriate ionic strength (Iurlaro and Muñoz-Pinedo, 2016; Lai *et al.*, 2007). The concentration of Ca^{2+} in ER is higher than that from the other cell compartments and Ca^{2+}-dependent chaperones, such as glucose-regulated protein 78 (Bip/Grp78), Grp94 and calreticulin holed in ER lumen. The Ca^{2+} concentration inside the ER lumen plays an essential role in controlling the activation of ER Ca^{2+}-dependent channels, ER homeostasis, endomembrane Ca^{2+} uptake and numerous enzymatic pathway (Burdakov *et al.*, 2005). The ER oxidative environment is vital for the formation of disulfide bonds by protein disulfide isomerase (PDI) 1 (Anelli and Sitia, 2008).

Very recently, in several papers it was reported that ER stress plays an important role in the progression and development of many diseases including type 2 diabetes, atherosclerosis, cancer, liver disease, respiratory diseases, and neurodegeneration (Ren *et al.*, 2021; Magallón *et al.*, 2021; Mustapha *et al.*, 2021; Li *et al.*, 2021; Naia *et al.*, 2021). Three UPR main signaling systems can be activated by three transmembrane sensor proteins, namely protein kinase RNA-like ER kinase (PERK), activating transcription factor-6 (ATF6) and inositol requiring protein-1 (IRE1) (Lai et al., 2007). These sensors are holed in their ineffective configuration and interact with the immunoglobulin heavy chain-binding protein (BiP/ or glucose-regulated protein 78 (Grp78). The

aggregation of unfolded and misfolded proteins in the ER lumen results in the release of BiP and UPR activation. The activation of PERK is through autophosphorylation. Thus, activated PERK phosphorylates the eukaryotic translation initiation factor 2 alpha (eIF2α) and causes a reduction in total translation exception of some proteins, such as activating transcription factor 4 (ATF4) that adjusts genes involved in ER homeostasis. PERK-ATF4 pathway activates C/EBPα-homologous protein (CHOP) that is a pro-apoptotic mediator and its downregulate DNA damage-inducible protein-34 (GADD34) gene (Urra *et al.*, 2013; Lenna *et al.*, 2014). After the separation of GRP78, ATF6 translocates from the ER to the Golgi body by proteolytic processing. This actives ATF6, and goes toward the nucleus to stimulate genes containing an ER stress response element (ERSE) in their promoter sequence. Several targets for ATF6 comprise the X box-binding protein 1 (XBP1), ER chaperone proteins (GRP78, GRP94), and the transcription factors CHOP and disulphide isomerase (Nadanaka *et al.*, 2007; Nadanaka *et al.*, 2004; Shen *et al.*, 2002). IRE1 is a type I ER transmembrane protein with serine/threonine kinase activity. It has endonuclease activity and can remove a 26-nucleotide sequence from the XBP1 mRNA formerly induced by ATF6. This spliced variant XBP1 (sXBP1) encodes an active transcription factor (Lee *et al.*, 2003). SXBP1 has various targets that comprise heat shock proteins (HSP)40 family member $P58^{IPK}$ and ER chaperones. $P58^{IPK}$ binds to PERK and prevents its activity, thereby creating a loop of negative feedback and decreases translational block caused by PERK-mediated (Yan *et al.*, 2002; Szegezdi *et al.*, 2006). Finally, after $P58^{IPK}$ gene silencing, the expression of both ATF4 and CHOP is enhanced (van Huizen *et al.*, 2003). IRE1 also initiates cell death through activation of kinase pathways; especially the c-Jun N-terminal kinase (JNK) pathway. Activated IRE1 recruit the TNF-receptor-associated factor 2 (TRAF2). Under ER stress conditions, the IRE1–TRAF2 forms a complex with the apoptosis-signal-regulating kinase (ASK1) (Szegezdi et al., 2006). ASK1

overexpression induces apoptosis in some cells (Hatai *et al.*, 2000). This pathway is a usual response to several forms of stress especially in the regulation of B-cell lymphoma 2 (BCL2) family proteins (Davis, 2000). However, PERK, ATF6 and IRE1 signaling do not directly cause cell death, but they initiate the activation of molecules including CHOP and JNK which lead to cell death. Both CHOP and JNK remove the BCL2 anti-apoptotic effect; JNK phosphorylates of BCL2 (and Bim), while CHOP blocks its expression. Eventually, these changes lead to the activation of the pro-apoptotic BAK and BAX, and mitochondria execute the cell death program (Szegezdi et al., 2006). All in all, therapeutic interventions (particularly natural compounds linke quercetin) that target molecules/signaling of the ER stress can be a promising strategy to treat related disorders.

3. Quercetin chemistry and pharmacokinetic properties

Quercetin (3,3',4',5,7-pentahydroxylflavone) is a plant-derived secondary metabolite which belongs to the flavonoids class (Murakami *et al.*, 2008; D'Andrea, 2015). More than 9,000 flavonoids have been identified so far (Hussain *et al.*, 2020). These are natural phenolic compounds of low molecular weights that contain a diphenylpropane (C6-C3-C6) skeleton and derive from the shikimic acid pathway. Flavonoids are classified into more than 10 groups, with flavanones, flavonols, flavanols, flavones, isoflavones and anthocyanins being the main groups present in the diet (Le Marchand, 2002). Such compounds are found in berries, tea, dark chocolate, *Citrus* fruits and red wine (Patel *et al.*, 2015). Quercetin itself is present in seeds, flowers, barks, nuts, and leaves of many plants, including apples, berries, onions, tea, and Brassica vegetables (Nathiya et al., 2014; Anand David *et al.*, 2016). It accounts for approximately 75% of the total flavonoid intake in the daily diet and in nature. It is conjugated within plants with sugar moieties (i.e. as glycosides), such as rutinose or rhamnose (Xiao *et al.*, 2018; Santangelo *et al.*, 2019).

Among the dietary flavonoids, quercetin glycosides have the best absorption in humans (Manach *et al.*, 2005). Quercetin is metabolized rapidly and excreted in the urine without accumulating in body tissues or fluids. It is believed that dietary quercetin is also excreted *via* the stools without absorption (<2%) but evidence shows that a remarkable amount (44.8%) of dietary quercetin is absorbed from the gastrointestinal tract and undergoes subsequent metabolic conversion (Murakami et al., 2008; Gugler *et al.*, 1975; Walle *et al.*, 2001). The main factors that influence the absorption of quercetin include its solubility and the nature of its conjugated sugar moiety (D'Andrea, 2015). Quercetin glycosides show the best absorption among all dietary flavonoids. They are rapidly absorbed from the gastrointestinal tract and reach plasma concentrations in less than a few hours (Manach et al., 2005). The plasma peak of quercetin glycosides is reached about 30 minutes after a one-time ingestion of 331 μmol (154 mg) of quercetin-4′-glucoside or 325 μmol (151 mg) quercetin-3-glucoside (Olthof *et al.*, 2000). Quercetin monoglucosides are rapidly hydrolyzed by lactose phlorizin hydrolase (LPH) at the brush border membranes of the small intestine. Other glycosides including monoglucosides (except glucose), disaccharides or oligosaccharides are deconjugated by colon bacteria and generate the corresponding quercetin aglycones. The latter are metabolized into sulfated and/or glucuronide derivatives *via* phase II metabolism. Quercetin 3-*O*-β-D-glucuronide is the major metabolite of quercetin in the blood and acts as a quercetin carrier (Xiao et al., 2018; Murakami et al., 2008). The degradation of quercetin in the colon is caused by the colonic microflora that break the C-ring in quercetin. Subsequent metabolites are absorbed through the colon epithelial cells and conjugated to sulfated and/or glucuronide, and finally be excreted in the urine or directly excreted in the feces (Almeida *et al.*, 2018). Thus, quercetin can be as a promising strategy to modulate ER stress in the pathophysiological development of diseases.

4. Quercetin biological activity

4.1 Anticancer activity

Quercetin has demonstrated anticancer activity through stimulation of the internal and external apoptosis pathways, and inhibition of transformation, mutagenesis, tumorigenesis and angiogenesis (Cruz *et al.*, 2008; Kraskiewicz and FitzGerald, 2012). Quercetin (20 μM) significantly increased the cytotoxicity of cisplatin in ovarian cancer C13* and P-ris cells and upregulated the expression of ER stress markers GRP78 and CHOP. It increased eIF2α phosphorylation and the expression of ATF4 in both cell types through activation of the PERK pathway. Furthermore, it induced splicing of XBP1 mRNA, which indicated the activation of IRE1 signaling (Yang et al., 2015b).

Another study on human ovarian cancer cells (OV2008) showed that quercetin increased the sensitivity of tumors to ionizing radiation (IR), *via* the PERK pathway by increasing CHOP-induced apoptosis and inducing the expression of p53 in a dose-dependent manner concomitantly (Gong *et al.*, 2018b). Quercetin also showed dose and time-dependent cytotoxicity towards human prostate cancer (PC-3) cells *via* cell cycle arrest and apoptosis. Quercetin decreased levels of cyclin D and E, Cdc25 and cyclin dependent kinase 2 (CDK2) but increased the levels of p18, p21, p27 and p53 related to cell cycle arrest in G0/G1 phase. On the other hand, quercetin reduced levels of Bcl-2, Bid, poly (ADP-ribose) polymerase (PARP) and pro-caspase-3 whereas it elevated the levels of apoptosis-inducing factor (AIF) and endonuclease G (EndoG), Bax, cytochrome C and caspase-9 associated with apoptosis. Likewise, quercetin induced the protein expression of growth arrest and DNA damage 153 (GADD153), ATF, and GRP78 which are hall markers of ER stress. Quercetin exposure led to apoptosis in PC-3 cells *via* disruption of the Ca^{2+} gradient (Liu *et al.*, 2014b). In human leukemia U937 cells, quercetin can reduce the expression of Hsp70. The latter

is expressed at low levels in normal cells and highly expressed in many type of tumors. Hsp70 binds to IRE1α and Hsp70- IRE1α complex permits the upregulation of BiP while not modifying CHOP upregulation. These results suggest that quercetin could be used as an effective adjuvant in combination with drugs causing ER stress in antileukemia therapy (Storniolo *et al.*, 2015a). Treatment of HCT-116 colon cancer cells with a synthetic derivative of quercetin (5,3′-dihydroxy-3,7,4′ -trimethoxyflavone or TEF) increased the levels of IRE1α and XBP-1, and reduced the levels of PERK and ATF-6 in a dose-dependent manner. The activation of ER stress proteins led to imbalanced levels of Ca^{2+}, which activates Ca^{2+} dependent enzymes (calpain) and the production of reactive oxygen species (ROS). Elevation of Ca^{2+} levels induced the JNK pathway which inhibited the function of BCL2. ROS production activated caspase-9 and caspase-3 which led to cell death (Khan *et al.*, 2016a). Quercetin, in the presence of aconitine, showed synergistic inhibition of multidrug resistance mutation 1 (MDR1) gene in HeLa cells in a dose-dependent manner. It also induced ER stress by upregulating the mRNA expression levels of eIF2α, CHOP and ATF4 (related to PERK pathway), XBP1 and GRP78 (related to IREI pathway) and ATF6 (related to ATF6 pathway) in HeLa cells. The production of ROS caused a significant decrease in the potential of the mitochondrial membrane (Li *et al.*, 2018a). These data suggest that quercetin may have properties to inhibit the development of cancer by the modulation of ER stress signaling pathways.

4.2 Effects of quercetin on liver and pancreas disorders

Quercetin (100 mg/kg for 35 days) significantly reduced the levels of aspartate/alanine transaminase (AST/ALT) and malondialdehyde (MDA), decreased ROS accumulation, and increased the levels of glutathione in mice. It decreased the mRNA expression of the inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6. The hepatoprotective effect

of quercetin has been linked to the inhibition of GRP78 which reduces the expression of IRE-1α and decreases the levels of inflammatory agents. It was suggested that the phosphoinositide 3-kinases (PI3K)/ nuclear factor kappa B (NF-κB) pathway played an important role in the observed effects during intense exercise/ER stress-triggered inflammatory injury in liver cells because the expression of hepatic PI3K and p-Akt levels were remarkably diminished (Tang *et al.*, 2016a). Administration of quercetin (10 μM), cyanidin, resveratrol or catechin to HepG2 hepatocytes treated with palmitic acid to increase ROS production and intracellular lipid accumulation prevented a reduction in the mitochondrial membrane potential ($\Delta\Psi$m). Collapse of the $\Delta\Psi$m plays a key role in apoptosis polyphenols have been shown to inhibit the decline in mitochondrial membrane potential. Palmitic acid elevated the mRNA expression of ER chaperones, such as GRP78, ORP150, GRP94 and of the co-chaperone ER-localized DnaJ homologue 4 (ERdj4) which are related to ER stress. Quercetin reduced the expression of four of the chaperones. Quercetin also significantly decreased the expression of the ER degradation-enhancing α-mannosidase-like protein (EDEM1), ATF4, and CHOP. EDEM1 is a marker of the IRE1 pathway. Quercetin (and resveratrol) increased the expression of the DNA-encoded mitochondrially NADH:ubiquinone oxidoreductase core subunit 1 (MTND1), improving the function of mitochondria. The mRNA expression of inducible nitric oxide synthase (iNOS), which increased with palmitic acid, was completely inhibited by quercetin and the other tested polyphenols. The product of Inos, NO, is well-known for facilitating the progression of liver steatosis (Rafiei *et al.*, 2018). The assembly and secretion of very low-density lipoproteins (VLDL) are vital for maintaining the plasma and hepatic homeostasis of lipids which are related to ER stress (Cohen *et al.*, 2011). It has been reported that quercetin significantly inhibited XBP1s which increased triacylglycerol of liver cells by decreasing the assembly of VLDL. Hepatic VLDL lipophagy and assembly are the major targets

of quercetin against non-alcoholic fatty liver disease through the IRE1a/XBP1s pathway. The reduction in NF-κB expression was observed following treatment with quercetin (Zhu *et al.*, 2018). In several studies, ER stress is activated *via* the NF-κB and inflammasome pathways (Tang et al., 2016a; Zhang and Kaufman, 2008; Glass and Olefsky, 2012). The effect of quercetin on ER stress were also investigated using an *in vitro* co-culture of pancreatic β-cells and endothelial cells. This study reported that quercetin (25 μM) could protect pancreatic ß-cells from ER stress *via* NO signaling. NO decreased the levels of phosphoproteins involved in the MAPK pathway such as pJNK/2, pp38 and pERK1, and those of the activating transcription factor-2 (pATF2) thereby preventing apoptosis. In addition, quercetin modulated the expression of CHOP (a negative regulator for NO signaling), thereby ameliorating endothelial nitric oxide synthase (eNOS) expression. It also reduced the levels of iNOS produced under ER stress (Suganya *et al.*, 2018d). Another study reported that the oral administration of quercetin (50 mg/kg for six weeks) decreased pancreatic ER stress-induced endothelial disorders and increased the levels of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in diabetic rats (Suganya *et al.*, 2018b).

In HepG2 cells treated with TNF-α to induce insulin resistance and ER stress, the administration of quercetin (3 and 5 μg/mL) led to a remarkable reduction in the expression of CHOP, IRE1α, XBP-1 and GRP78 compared with the TNF-α-induced control group. Quercetin also significantly decreased the phosphorylation of JNK and IRS-1, and the expression of gluconeogenic genes (Park *et al.*, 2018). Collectively, these findings indicated that the application of quercetin could be useful in the treatment of such diseases *via* modulation of ER stress.

4.3 Effect of quercetin on the central nervous system (CNS) and peripheral nervous system (PNS)

Alzheimer's disease (AD) is a cognitive neurodegenerative disease that includes the presence of extracellular senile plaques formed by amyloid-β (Aβ) protein, and intracellular neurofibrillary tangles, as two histopathological markers (Morris and Tangney, 2014). In a study on aged APP23 transgenic mice, quercetin increased the expression of GADD34 which reduces the expression of ATF4 and led to a decrease in eIF2α phosphorylation. Quercetin also suppressed the expression of presenilin-1 and Aβ secretion through GADD34 induction. Presenilin-1 is an aspartate protease involved in the formation of Aβ plaques. These results suggest that quercetin could delay the deterioration of memory at the primary stage of AD (Hayakawa *et al.*, 2015). In another study on human neuroblastoma (SH-SY5Y) cells, quercetin and quercetin-3-*O*-glucuronide (Q3G) inhibited the phosphorylation of tau protein by preventing the activation of glycogen synthase kinase 3β (GSK3 β) *via* increasing phosphorylation at Ser 9 residue. Hyperphosphorylation of tau protein is responsible for neuronal damage in AD and activation of GSK-3β enhances tau hyperphosphorylation. Quercetin and Q3G used the AMP-activated protein kinase (AMPK) pathway for the prevention of tau phosphorylation that effectively decreased IL-6 and IL-1 β production in neuronal cells. In addition, quercetin decreased the expression of P-IRE1α and P-PERK, regularized NLR family, pyrin domain containing 3 (NLRP3) and inhibited ER stress/NLRP3 inflammasome activation in the hippocampus. Finally, quercetin as well as Q3G prevented the reduction in ΔΨm in neuronal cells (Chen *et al.*, 2016). Pre-treatment with quercetin (50 μM for 24 h) before gamma radiation (2 Gy) significantly decreased BiP and CHOP expression in irradiated dorsal root ganglion neurons. The results also showed that quercetin downregulated the expression of TNF-α, pJNK and JNK which reduced the release of pro-inflammatory cytokines. Likewise, quercetin significantly elevated the expression of Tuj1 (neuron-specific class III beta-tubulin) contributing to microtubule stability in the neuronal cell and reducing ER stress

(Chatterjee *et al.*, 2019b). Oxidative and nitrosative stress can lead to ER stress (Zhao *et al.*, 2012). INOS gene expression is adjusted by the transcription factors in the NF-*κ*B signaling family specially p65. It has been reported that treatment with quercetin-3-*O*-glucoside decreased p65 levels in the embryos of diabetic mice. That compound also significantly reduced the expression of BiP, P-IRE1α and P- eIF2α which are ER stress markers (Tan *et al.*, 2018).

Thus, quercetin could be as a promising candidate to reduce ER stress, regulating inflammation and subsequently the pathogenesis and progression of neurodegenerative diseases

4.4 Effect of quercetin on the cardiovascular system

Quercetin has been demonstrated to decrease the production of ROS and inhibit the NOD-like receptor family, pyrin domain containing-3 (NLRP3) and thioredoxin-interacting protein (TXNIP) inflammasome activation induced by palmitate. NLRP3 and TXNIP promote induction of IL-1 β and IL-6 that can lead to ER stress. Quercetin can also elevate basal AMPK activity through enhancing phosphorylation of AMPK leading to enhanced NO production in endothelial cells, and prevent cell apoptosis *via* changes in ΔΨm (Wu *et al.*, 2015).

The inhibitory effect of 5,7-dideoxyquercetin or 3′,4′-dihydroxyflavonol (DHF) on ER stress induced by tunicamycin was investigated in the aortae of C57BLK/6 J mice. DHF reduced the expression of CHOP and GRP78, eIF2α phosphorylation and caspase-3 cleaved form. In addition, DHF reduced ROS production and elevated the levels of NO in aortic rings. An *in-vitro* study showed that DHF decreased GRP78 expression and eIF2 α phosphorylation that caused an increase of XBP1 splicing and led to apoptosis (Lau, Yeh Siang *et al.*, 2018). Oral administration of quercetin (10 mg/kg/day) was shown to prevent the progression of experimental autoimmune myocarditis (EAM) to dilated cardiomyopathy (DCM). Quercetin decreased the myocardial

311 expression of GADD153 and GRP78. Furthermore, it significantly suppressed the myocardial
312 MAPK and endothelin-1 (ET-1) that caused the progression of EAM. Quercetin decreased the
313 expression of TGF-ß 1 and mouse polyclonal anti-osteopontin (OPN) that occur in fibrosis. It also
314 reduced the cytosolic cytochrome C level which are elevated in apoptosis (Arumugam *et al.*,
315 2012b). Thus, based on these studies, quercetin can be useful to reduce the effect of ER stress
316 signaling pathways and protect against cardiovascular disease.
317

318 **4.5. Effect of quercetin on other organs**

319 The results of an investigation on human colonic LS180 cells showed that quercetin inhibited the
320 induction of GRP78 expression by ER stressors including tunicamycin, A23187 and thapsigargin
321 at both the protein and mRNA levels. This effect was reproduced by wortmannin and LY294002
322 which are PI3K inhibitors but neither by vitamin E nor vitamin C. Therefore, quercetin inhibited
323 the ER stress created by Ca^{2+} dynamics dysregulation through PI3K inhibition. Quercetin also
324 suppressed the induction of PERK and IRE1 by thapsigargin or A23187 although it activated
325 PERK and IRE1 when added to LS180 cells alone (Natsume *et al.*, 2009). It has reported that
326 zearalenone and its metabolites (α-zearalenol and β-zearalenol) produced ER stress and induced
327 the UPR through XBP1 mRNA splicing and increase of ATF4, GRP78, GADD34 and CHOP in
328 human colon carcinoma cells (HCT116 cells). Quercetin (5 mM) pretreatment significantly
329 reduced the expression of the GRP78 ER stress chaperone and decreased GADD34 induction.
330 Moreover, pretreatment with quercetin (5 mM) significantly diminished the level of ROS (Ben
331 Salem *et al.*, 2016b).
332 The protective effect of quercetin on titanium particles that induce ER stress and osteolysis was
333 studied in BALB/C mice and murine macrophage cell line RAW264.7 models. It was shown that

quercetin remarkably decreased titanium particle-induced amplification of the expression levels of GRP78, CHOP, PERK, IRE1, caspase-3 and caspase-12 and increased the down-regulation of Bcl-2 in both models. Quercetin also decreased IL-1β, TNF-α and IL-6 release from RAW264.7 cells which demonstrated its anti-inflammatory effect (Zhang *et al.*, 2017b). In another study on RAW264.7 macrophages, quercetin significantly suppressed the activation of IRE1, ATF6 and XBP1 and decreased the expression of CHOP (Yao *et al.*, 2012). A summarizing of the beneficial effects of quercetin along with doses, the model, pharmacological effects and mechanisms are shown in table 1.

5. Quercetin modes of delivery

The main challenge in using quercetin for therapeutic purposes is the fact that it has a low efficacy because of limited aqueous solubility, and bioavailability. Various formulations and drug delivery systems (e.g. microspheres emulsions, conjugates, biomimetic/metallic nanoparticles) have been designed to overcome these issues, in particular to enhance the permeation of quercetin across the blood–brain barrier (Parhi *et al.*, 2020; Liu *et al.*, 2020; Chakraborty *et al.*, 2012; Guan *et al.*, 2021) (Table 2). These advanced quercetin delivery programs improve its effects on targets.

6. Conclusions and future prospects

In this review, we have highlighted the various pathways involved in the activation of the ER stress response that can be regulated by quercetin (Figure 2). Although quercetin has a low absorption rate and bioavailability, the various new formulations and delivery systems that have recently been developed have the potential to overcome these limitations. Studies carried out to date suggest that quercetin could be used in combination with conventional anticancer treatments to strengthen the effects of chemotherapy. Further *in-vivo* and clinical studies are warranted to confirm the beneficial effects of quercetin.

Conflict of interest

The authors declare no conflict of interest.

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Data Availability Statement

Not applicable.

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630 **Table 1**. The beneficial effects of Quercetin; dose, the model, pharmacological effects and mechanisms.

Compound	Dose/Concentration, Time	Model/Subject	Pharmacological effects	Mechanisms	Reference
Quercetin	20 μM, 12-24 h	*in vitro*/ ovarian cancer C13* and P-ris cells	↑ Cytotoxicity of cisplatin	↑ expression GRP78 and CHOP ↑ eIF2α phosphorylation and the expression of ATF4	(Yang *et al.*, 2015a)
Quercetin	100 μM,12 and 24 h	*in vitro*/ human ovarian cancer cells (OV2008)	↑ Sensitivity of tumors to ionizing radiation (IR)	↑ CHOP-induced apoptosis and inducing the expression of p53	(Gong *et al.*, 2018a)
Quercetin	50-200 μM, 24 and 48 h	*in vitro*/ human prostate cancer (PC-3) cells	Cytotoxic effects	↓ cyclin D and E, Cdc25 and CDK2 ↑ p18, p21, p27 and p53	(Liu *et al.*, 2014a)

				↓ Bcl-2, Bid, PARP and pro-caspase-3 ↑ AIF, EndoG, Bax, cytochrome C and caspase-9 ↑ GADD153, ATF, and GRP78	
Quercetin	10 μM, 30 min	*in vitro*/ human leukemia U937 cells	Antileukemia effects	↓ expression of Hsp70	(Storniolo *et al.*, 2015b)
5,3′-dihydroxy-3,7,4′ -trimethoxyflavone (TEF)	25 μM and 50 μM, 24 h	*in vitro*/ colon cancer cells (HCT-116)	Apoptotic cell death of colon cancer cells	↑ IRE1α and XBP-1 ↓ PERK and ATF-6 ↑ production of ROS and activity of caspase-9 and caspase-3	(Khan *et al.*, 2016b)
Quercetin	44.08 μg/mL, 24 h	*in vitro*/ HeLa cells	Synergistic inhibition of MDR1 gene	↑ eIF2α, CHOP and ATF4, XBP1, GRP78 and ATF6	(Li *et al.*, 2018b)
Quercetin	100 mg/kg, 35 days	*in vivo*/ male adult BALB/C mice	Hepatoprotective effects	↓ AST/ALT, MDA and ROS ↓ TNF-$\alpha$and IL-6 inhibition of GRP78 which reduces the expression of IRE-1α	(Tang *et al.*, 2016b)
Quercetin	10 μM, 2 h	*in vitro*/ HepG2 cells	Hepatoprotective effects against Palmitic acid	↓ GRP78, ORP150, GRP94 and of the co-	(Rafiei et al., 2018)

				chaperone ERdj4 ↓ EDEM1, ATF4, and CHOP ↓ iNOS activity and NO production	
Quercetin	25 μM, 2 h	*in vitro*/ pancreatic β-cells	Pancreato-protective effects	↓ expression of CHOP ↓ eNOS and iNOS activity	(Suganya *et al.*, 2018c)
Quercetin	50 mg/kg, six weeks	*in vivo*/ Male albino Wistar rats	Improving diabetes-induced endothelial dysfunction	↓ Pancreatic ER stress-induced endothelial disorders ↑ SOD, catalase and GPx ↑ VEGF and VEGFR2 expression ↓ serum nitrite and serum cGMP	(Suganya *et al.*, 2018a)
Quercetin	3 and 5 μg/mL, 24 h	*in vitro*/ HepG2 cells	Protective effects against TNF-α-induced ER stress and insulin resistance	↓ Serine phosphorylation of IRS-1, phosphorylation of JNK, and the expression of gluconeogenic genes ↓ expression of CHOP, IRE1α, XBP-1 and GRP78	(Park et al., 2018)

Quercetin	50 μM, 12 h	*in vitro* / autophagy impaired Atg5KD/SC100/HEK293 cells	Neuroprotective effects against Alzheimer's disease	↑ GADD34 ↓ ATF4 and eIF2α phosphorylation ↓ Presenilin-1 and Aβ secretion	(Hayakawa et al., 2015)
Quercetin Quercetin-3-*O*-glucuronide	10 μM, 6 h	*in vitro* / human neuroblastoma (SH-SY5Y) cells	Neuroprotective effects against Alzheimer's disease	↑ AMPK activity ↓ IRE1α and PERK phosphorylation, NLRP3 expression and tau phosphorylation ↑ Mitochondrial membrane potential (ΔΨm)	(Chen et al., 2016)
	50 mg/kg, 10 weeks	*in vivo*/ Male C57BL/6J mice			
Quercetin	25 μM and 50 μM, 24 h	*in vitro* / primary cultured dorsal root ganglion (DRG) neurons	Neuroprotective role against radiation-mediated ER stress	↓ expression of BiP and CHOP ↓ expression of TNF-α, pJNK and JNK ↑ Tuj1	(Chatterjee *et al.*, 2019a)
Quercetin	10 μM, 1 h	*in vitro* / Human Endothelial Cells (EA.hy926)	Protective effects against endothelial dysfunction	↑ AMPK activity ↓ NLRP3 and TXNIP ↓ IL-1β and IL-6 ↑ Mitochondrial membrane potential (ΔΨm)	(Wu *et al.*, 2014)
5,7-dideoxyquercetin or 3',4'-	150 mg/kg/day, two weeks	*in vivo*/ Male C57BLK/6 J mice	Protective effects against vascular injury	↓ CHOP and GRP78, eIF2α phosphorylation	(Lau, Y. S. *et al.*, 2018)

dihydroxyflavonol (DiOHF)				and caspase-3 cleaved form ↓ ROS production and elevated the levels of NO	
Quercetin	10 mg/kg/day, 28 days	*in vivo*/ Male Lewis rats	Cardioprotective effects against autoimmune myocarditis	↓ TGF-β ↓ GRP78, GADD and cytochrome-C ↓ ET-1, phospho p38 MAPK and phospho ERK	(Arumugam *et al.*, 2012a)
Quercetin	25-150 μM, 24 h	*in vitro*/ human colonic LS180 cells	Protective effects against dysregulation of calcium dynamics	↓ PERK, IRE1 and PI3K ↓ GRP78 and phosphorylation of eIF2	(Natsume et al., 2009)
Quercetin	5 μM, 2 h pretreatment	*in vitro*/ human colon carcinoma cells (HCT116 cells).	Protective effects against Zearalenone toxicity	↓ GADD34, GRP78, ATF4 and CHOP ↑ Mitochondrial membrane potential ($\Delta\Psi$m) ↓ caspase 3 activity	(Ben Salem *et al.*, 2016a)
Quercetin	40, 80, and 160 μM, 30 min	*in vitro*/ RAW264.7 cells	Protective effects against osteolysis	↓ GRP78, CHOP, PERK, IRE1, caspase-3 and caspase-12 ↑Bcl-2 ↓ IL-1β, TNF-α and IL-6 release	(Zhang *et al.*, 2017a)

Table 2: Novel systems developed to enhance the delivery of quercetin

Formulation	Disorder	Experimental model	Result	Reference
Nano-encapsulation	Gastric ulcer	*In-vivo*	Protection of mitochondrial integrity, size, and mitochondrial functions	(Chakraborty et al., 2012)
Nano-encapsulation	Cardiac diseases	*In-vitro*	Improved cardioprotection during hypoxia-reperfusion injury *via* preservation of mitochondrial function.	(Lozano *et al.*, 2019)
QTiO2	Fibroblast model	*In-vivo*	Improved bioavailability and stability of quercetin in cells, with maximum antioxidant ROS potency.	(Birinci *et al.*, 2020)
Qc@SNPs-MB	Alzheimer's disease	*In-vitro &In-vivo*	Improved crossing of drugs across the BBB. Fast accumulation of Qc@SNPs in the brain, effectively decreasing apoptosis, inflammation and oxidative stress.	(Liu et al., 2020)
N1QC	Cerebral ischemia–reperfusion	*In-vivo*	Decreased age-related global moderate cerebral ischemia reperfusion injury	(Ghosh *et al.*, 2017)
Quercetin loaded biomimetic nanoparticles	Tumor	*In-vitro* & *in-vivo*	Enhanced tumor targeting and the effects of radiotherapy	(Huang *et al.*, 2020)

Qc@SNPs-MB: Quercetin-modified sulfur nanoparticles (Qc@SNPs) in microbubbles (MB);

QTiO2: Quercetin in the form of a nano-antioxidant; **N1QC**: Quercetin loaded polymeric nanocapsules.

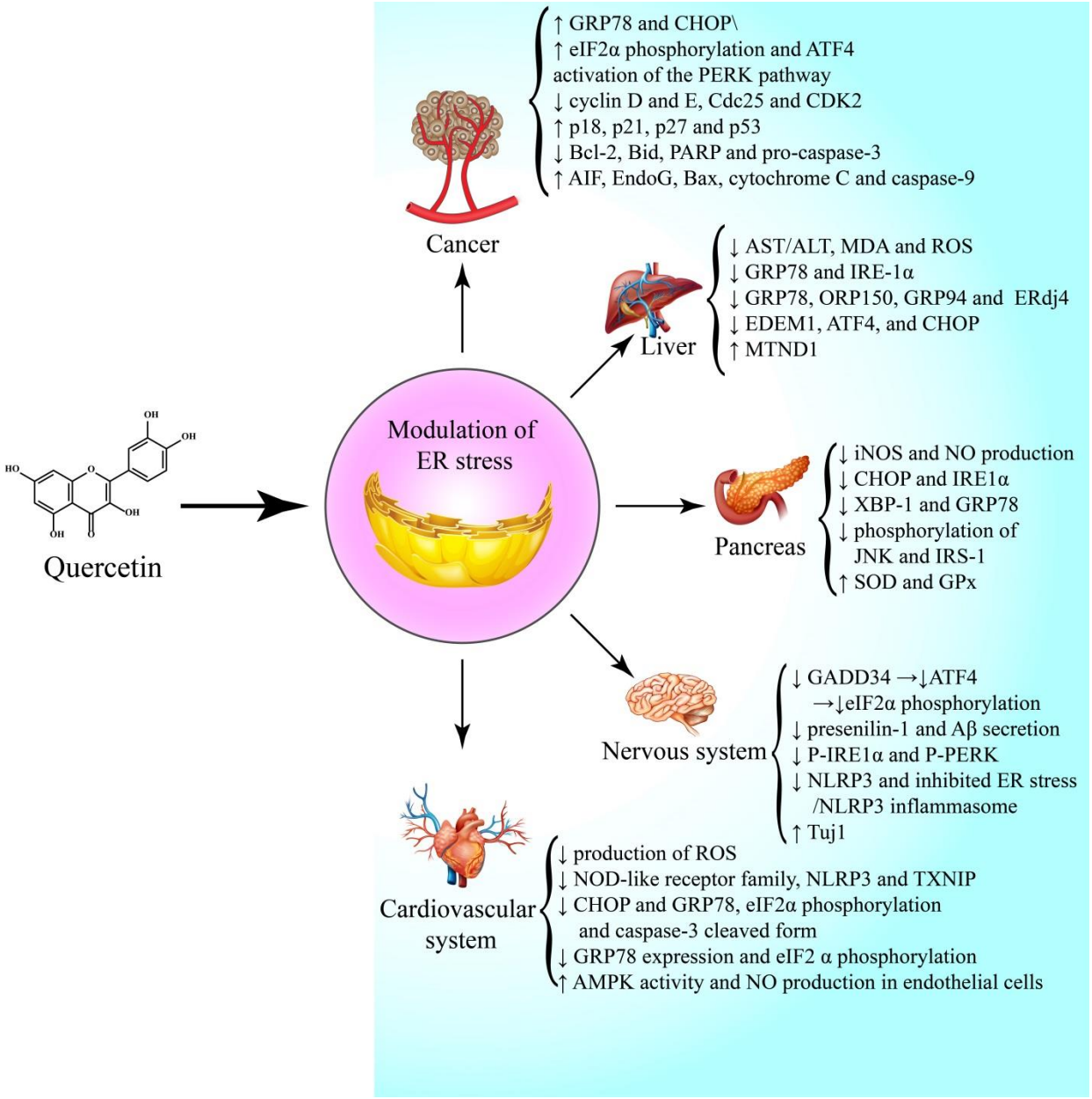

Figure 1. The role of ER stress in the various diseases

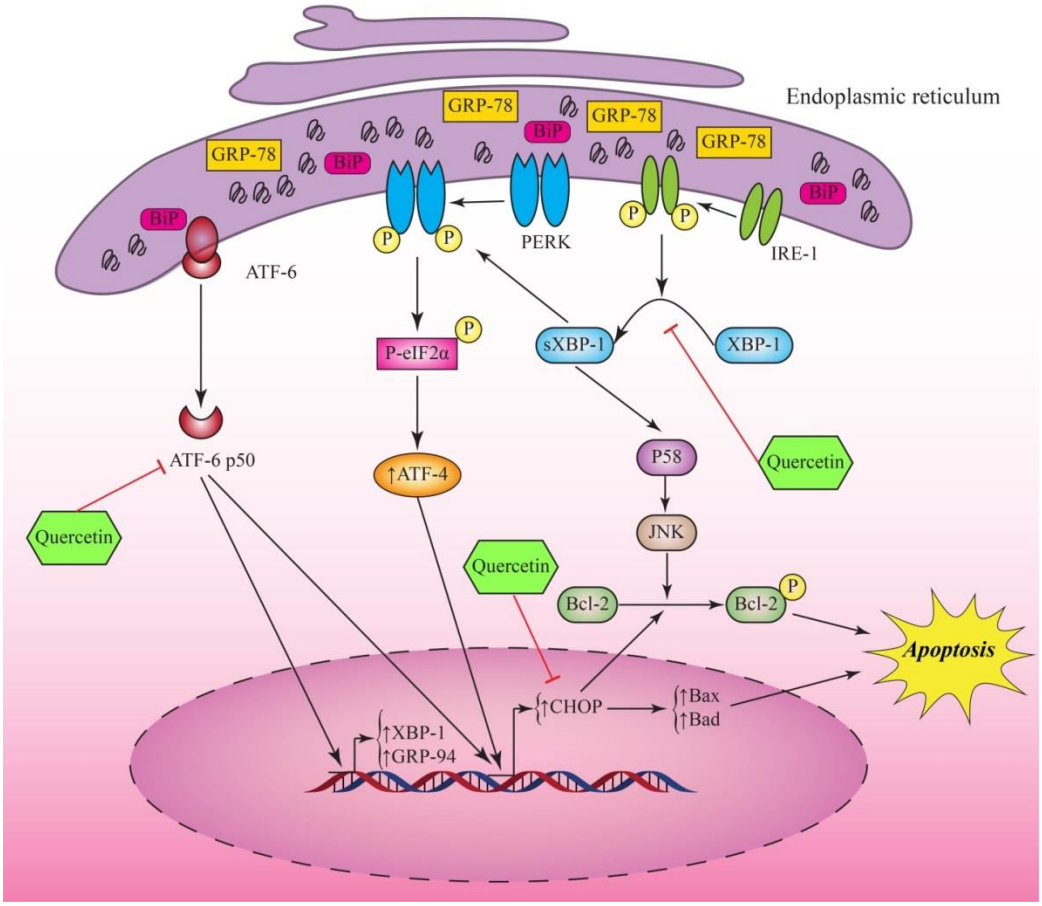

Figure 2. Cellular pathways involved in endoplasmic reticulum (ER) stress response activation, regulated by quercetin. **GRP**: Glucose-regulated protein; **PERK**: Protein kinase RNA-like ER kinase; **IRE1**: Inositol requiring protein-1; **ATF-6**: Activating transcription factor-6; **P-eIF2a**: Phosphorylation of the eukaryotic initiation factor 2; **sXBP-1**: Spliced form of X-box binding protein 1; **CHOP**: C/EBPα-homologous protein.