Cell and Tissue Research
https://doi.org/10.1007/s00441-022-03664-3

REVIEW

Exploring Nrf2 as a therapeutic target in testicular dysfunction

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Received: 16 March 2022 / Accepted: 27 June 2022

Abstract

Testicular dysfunction, a major contributory factor to infertility, has received a lot of attention over the recent years. Several studies have linked abnormal sperm function and morphology with an enhanced generation of reactive oxygen species (ROS) and oxidative stress. The nuclear factor erythroid-derived 2 (Nrf2) is a transcriptional response to cellular stresses (intrinsic or extrinsic) that regulates the oxidative status, mitochondrial dysfunction, inflammation, and proteostasis. In this review, the therapeutic role of Nrf2 was explored. To do so, scientific data were retrieved from databases such as Elsevier, Wiley, Web of Science, Springer, PubMed, Taylor and Francis, and Google Scholar using search terms such as "Nrf2" and "testis," "sperm," "testicular function," and "testosterone." It has been noted that Nrf2 influences the physiology and pathology of testicular dysfunction, especially in the spermatogenic process, by regulating cellular resistance to oxidative stress, inflammation, and environmental toxicants. However, numerous compounds serve as activators and inhibitors of testicular Nrf2. Nrf2 activators might play a therapeutic role in the prevention and treatment of testicular dysfunction, while molecules that inhibit Nrf2 might induce dysfunction in testis components. Nrf2 activators protect cells against oxidative damage and activate Nrf2/KEAP1 signaling which promotes its movement to the nucleus, and increased Nrf2 function and expression, along with their downstream antioxidant gene. Nrf2 inhibitors facilitate oxidative stress via interfering with the Nrf2 signal pathway. The Nrf2 activation could serve as a promising therapeutic target for testicular dysfunction. This review explored the effect of Nrf2 on testicular function while highlighting potential activators and inhibitors of Nrf2.

Keywords Nrf2 · Oxidative stress · Sperm · Testicular dysfunction · Oxidative stress · Cellular stresses

Introduction

The declining rate of fertility globally is rapid, and, for this reason, decreased sperm count and quality have been partly implicated. Indeed, several studies have linked a declining rate of fertility with decreased sperm quality and attribute it to testicular dysfunction (Kayode et al. 2020; Olaolu et al. 2018). Studies revealed that in the last 35 years, there has been a 57% reduction in sperm concentration around the globe, while Europe and Africa recorded 32.5% and 72.6% fertility declines in the past 50 years (Dutta et al. 2019; Sengupta et al. 2017). Several factors, including an increased amount of, and exposure to, chemical and physical agents, have resulted in sperm decline and ultimately attribute to testicular dysfunction (Bieniek et al. 2016).

In the testis, elevated reactive oxygen species (ROS) levels produce deleterious effects on sperm quality and function. ROS are crucial intracellular signaling molecules, but they could be detrimental to the organisms if produced in excess to overwhelm the antioxidant defense system (Dutta et al. 2019). The resulting condition is known as oxidative stress (OS), and several research studies have associated oxidative stress with testicular dysfunction (Dutta et al. 2019; Sabeti et al. 2016). It has been proposed that Nrf2 is important in avoiding oxidative disruption of spermatogenesis (Wajda et al. 2016). Testicular oxidative stress leads to the downregulation of the *Nrf2* gene and also increased testicular lipid peroxidation, germ cell death, and depleted antioxidant levels (Moreno-Fernandez et al. 2019).

Nrf2 protects the testis from oxidative stress by playing a critical role in cellular antioxidant defense (Wajda et al.

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Published online: 05 July 2022

2016). Nrf2 regulates the antioxidant response pathway in the promoters of antioxidant genes by binding to antioxidant response elements (AREs). These AREs are cis-acting enhancer sequences. Nrf2 is a member of the cap-n-collar family of basic leucine zipper (bZIP) proteins. Nrf2 binds normally with Kelch-like ECH-Associated Protein 1 (KEAP1) in the cytosol and targets Nrf2 degradation. Due to oxidative stress or electrophile presence, there is a modification of KEAP1 on its nucleophilic cysteine sulfhydryl groups, which causes an allosteric conformational change that diminishes the KEAP-dependent degradation of Nrf2 and also the Nrf2 accumulation in the nucleus (Kumar et al. 2014).

Nrf2 with the coordinated genes of the Nrf2 system is ubiquitously expressed in the testis and is an important defense against testicular toxic insults (Shahat et al. 2020). They are important in regulating the blood-testis barrier and testicular tissue function (Wajda et al. 2016). These coordinated genes are Nrf2-related genes that contain AREs in their promoters: Nrf2, HO-1, GCLC, and NQO1 (Cuadrado et al. 2019; Li et al. 2014; Saha et al. 2020). Furthermore, reduced Nrf2 expression in mouse testis and human sperm cells is linked to spermatogenesis dysfunction caused by diabetes and smoking (Jiang et al. 2014; Yang et al. 2019b). The NRF2 protein was found in the cytoplasm of spermatids, and it was also found in other germ cells (Jiang et al. 2014). Thus, Nrf2 may serve as a useful biomarker in the prediction of male infertility. Therefore, this review seeks to explore Nrf2 as a therapeutic target for testicular dysfunction.

Nrf2

Nrf2 is a cellular transcriptional response against oxidative stress ions by positively regulating the gene expression encoded by antioxidants, drug efflux pumps, and enzyme detoxification of xenobiotics (Loboda et al. 2016; Wu et al. 2019). Nrf2 regulates intracellular redox equilibrium by upregulating several downstream genes, effectively protecting the cell from harm caused by environmental insults (Sinha et al. 2013). Endogenous antioxidants enzymes such as glutathione peroxidase, heme oxygenase-1 (HO-1), thioredoxin reductase (TrxR), thioredoxin (Trx), peroxiredoxin, glutamate-cysteine ligase (GCL); drug transporters (multidrug resistance-associated protein-MRP); and phase II detoxifying enzymes such as glutathione S-transferase. Many *Nrf2* target genes have one or more AREs in their 50-flanking regions, including the prototypic inducible genes *GSTA1* and *NQO1*. Interacting partners such as Nrf2, ARE, and KEAP1 are involved in the Nrf2 signaling cascade, which is responsible for detecting and transducing stress chemical signals (Bryan et al. 2013; Kitamura and Motohashi 2018). In the Nrf2 signaling cascade, the controller is Nrf2, the sensor is KEAP1, and, finally, the responder is ARE (Uddin et al. 2020).

Genomic analyses showed that Nrf2-linked genes provide direct antioxidants; enhance the levels of synthesis and regeneration of glutathione; activate the synthesis of NADPH; increase toxin export through the multidrug-response transporters; encode enzymes that directly inactivate oxidants, receptors, and growth factors; catalyze the detoxification of xenobiotics, especially carcinogens; and increase the identification, repair, and elimination of damaged proteins and molecular chaperones (Hayes and Dinkova-Kostova 2014; Tonelli et al. 2018).

The structure of Nrf2 and KEAP1

The "Cap 'N' Collar" in the leucine zipper factor family provides the *NFE2L2* gene which encodes Nrf2. The NRF2 protein in humans contains 605 amino acids, and the NRF2-ECH homology (NEH) domains contain seven (7) conserved Neh1-Neh7. At the N-terminus of Nrf2 is Neh2, which serves as a major regulatory domain with two binding sites (DLG and ETGE). The interaction between these sites and the Kelch domains of the E3 ubiquitin ligase KEAP1 helps to regulate the stability of Nrf2 (Moon and Giaccia 2015; Tonelli et al. 2018). This stability can also be regulated by the Neh1 and Neh6 domains. Neh1 is a basic leucine zipper motif responsible for the promotion of Nrf2 transcriptional activation. Nrf2 stability is negatively regulated via the Neh6 domain, which is a serine-rich domain with two motifs (DSAPGS and DSGIS). The Nrf2 transactivation domains are the Neh3, Neh4, and Neh5 domains. The Neh3 domain binds to a chromo-ATPase/helicase DNA-binding protein (CHD6) at the carboxy-terminal, while the Neh4 and Neh5 domains interact with the CBP (CREB-binding protein) domains (Bhakkiyalakshmi et al. 2015; Ojo et al. 2017). A structural representation of the Nrf2 structure is shown in Fig. 1.

Regulation of Nrf2 activation

The Kelch/double glycine repeat domain and the IVR domain, which mediate the KEAP1 protein's binding to the actin cytoskeleton and contain a nuclear export signal, determine KEAP1 protein localization (Keum and Choi 2014; Zang et al. 2020). The nuclear localization signal on the NRF2 protein aids its translocation from the cytoplasm to the nucleus. The NRF2 protein binds to ARE to form a dimer with small MAF proteins in the nucleus (Fig. 2). Before Nrf2 binds to the ARE, it forms heterodimers with ATF4 or other bZIP transcription factors (Rössler and Thiel 2017).

As a consequence, sequestration and further depletion of Nrf2 in the cytoplasm are the pathways for the repressive impact of Keap1 on the function of Nrf2. The ability of such

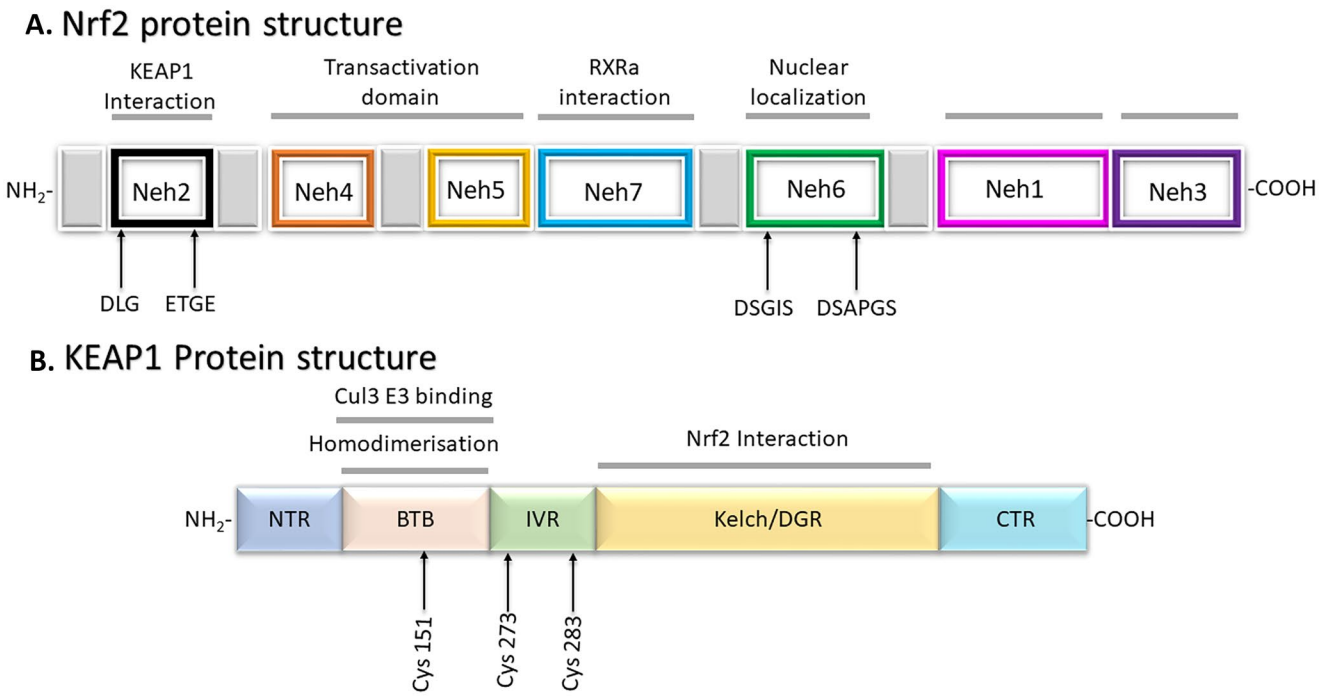

Fig. 1 Structural representation of Nrf2 and KEAP1. **A** The NRF2 protein contains six domains, Neh1-Neh6. **B** KEAP1 with its functional domains: the BTB, IVR, and the Kelch/β-propeller domains

triggers to repress KEAP1-dependent depletion is thought to be the driving force behind Nrf2's increased stability and activation. On exposure to both endogenous and exogenous Nrf2 inducers (chemo-preventive agents and heavy metals), the Nrf2–KEAP1 complex dissociates, rescuing Nrf2 from proteasomal degradation and significantly increasing its half-life (Krajka-Kuźniak et al. 2017; Sihvola and Levonen 2017). In response to stress, the Kelch-repeat domain binds the nuclear protein prothymosin-α to release Nrf2, which activates its target genes.

In response to inducers, two general pathways for Nrf2 nuclear accumulation have been proposed. The first is Nrf2 ubiquitination downregulation, which happens when KEAP1 cysteine thiols are modified or Nrf2 is phosphorylated or both. This causes Nrf2 to dissociate from KEAP1 by disrupting the KEAP1–Nrf2 and KEAP1–CUL3 complexes (Ishitsuka et al. 2020; Yamamoto et al. 2018). The second mechanism involves interfering with Nrf2's nuclear import and export. Several effectors mediate Nrf2 release from KEAP1 and subsequent translocation to the nucleus from the cytoplasm under redox stress. The effectors include KEAP1, protein kinase C (PKC)-mediated Nrf2 phosphorylation at Ser40, glycogen synthase kinase-3b (GSK-3b), phosphoinositide-3-kinase (PI3K), casein kinase 2 (CK2), c-Jun N-terminal kinase 1 (JNK1), extracellular signal-regulated kinase 2 (ERK2), and ERK5 and PKR-like ER-localized eIF2a kinase (PERK). The activation and phosphorylation of Nrf2 involve phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), and RNA-dependent protein kinase-like endoplasmic reticulum kinase (PERK). The CBP coactivator may be used by the ERK and JNK pathways to upregulate the Nrf2 domain (Pallesen et al. 2018; Tong et al. 2015).

Nrf2 activators as a potential therapeutic strategy for testicular dysfunction

Nrf2 activators have been shown to target KEAP1 and thus inhibit it. The activators of Nrf2 can be classified as electrophiles, multi-target drugs, and protein–protein interaction (PPI) inhibitors. Electrophilic molecules have been reported to have the most pharmacological activators of Nrf2. They modify, via oxidation or alkylation, the cysteine residues (mostly Cys-151, Cys-226, Cys-273, Cys-288, Cys-434, and Cys-613) that are present in the thiol-rich KEAP1 protein. Also, KEAP1 can be inhibited through its CUL3/RBX1 complex interaction that is required for the ubiquitination of Nrf2 (Fig. 2). Several compounds known to serve as activators of Nrf2 in the testis are listed in Table 1. Because targeting the Nrf2 signaling pathway has a crucial function in several mechanisms such as antioxidant metabolism, inflammatory modulation, and biotransformation reactions, Nrf2 activators are chemoprevention agents. To summarize, these

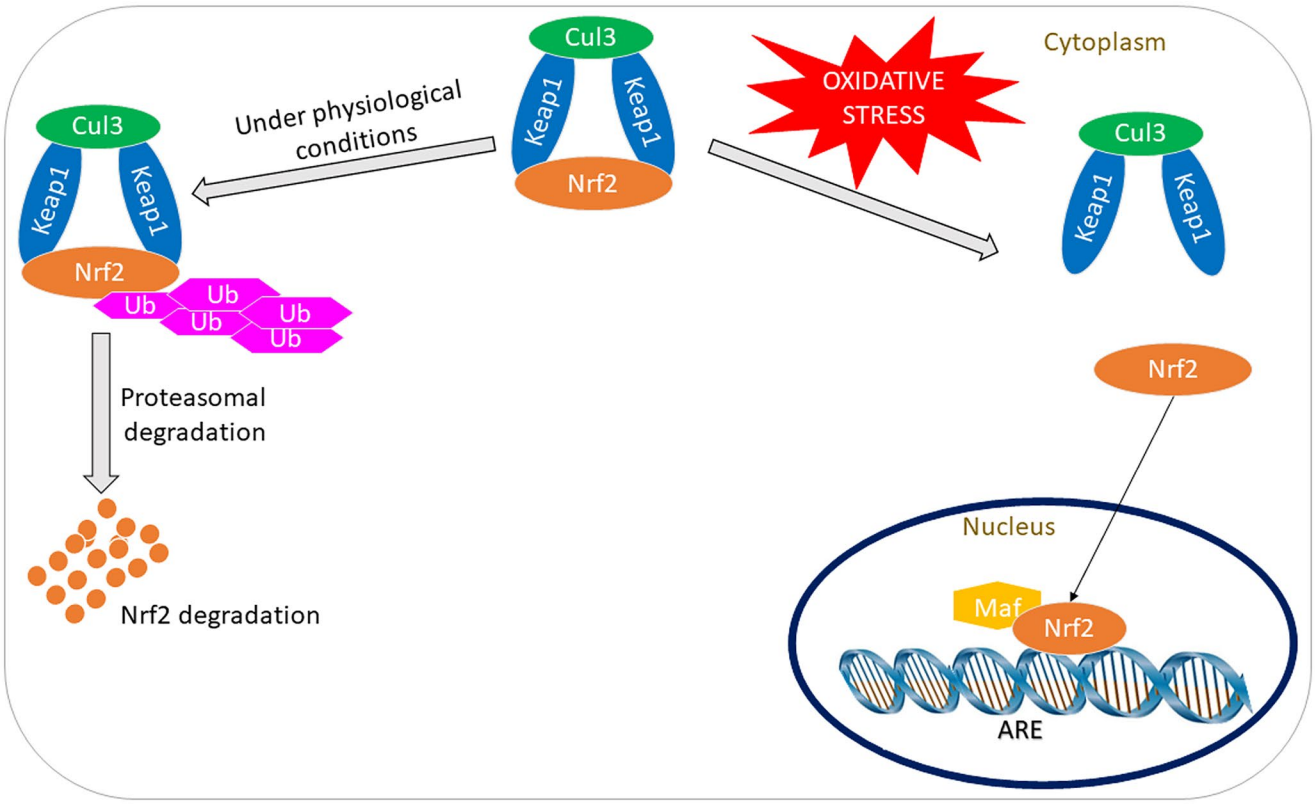

Fig. 2 Regulation of Nrf2. Under physiological conditions, Nrf2 is restricted in the cytoplasm via its association with the KEAP1-CUL3 complex, but under stressful conditions, Nrf2 is released from KEAP1

activators activate the Nrf2 pathway by four mechanisms: (i) Keap1 inhibition, (ii) Nrf2 nuclear translocation enhancement, (iii) Nrf2 mRNA and protein expression promotion, and (iv) Nrf2 upstream mediator activation. As a result, addressing this mechanism for the treatment of testicular dysfunction caused by its deregulation could be beneficial.

MitoQ is a mitochondrially targeted antioxidant made up of coenzyme Q10 and triphenylphosphonium (TPP+). MitoQ's excellent antioxidant capacity has been attributed to its protective role in several diseases such as diabetes, kidney, and neurodegenerative diseases (Sun et al. 2017; Xiao et al. 2017). Xiao et al. (2017) reported that MitoQ treatment in testis tissue enhanced Nrf2 expression, decreased KEAP1 expression, and therefore promoted Nrf2 nuclear translocation. This showed that MitoQ activates Nrf2/KEAP1 signaling, which promotes its movement to the nucleus and regulates Nrf2 level at the posttranslational level to mitigate oxidative stress.

Sulforaphane (SFN) is an isothiocyanate derivative that acts as a strong Nrf2 activator. In several oxidative stress conditions, SFN has protected cells against oxidative damage and activated Nrf2/ARE pathways, increased the expression and function of Nrf2, and increased the mRNA and protein expression of the downstream antioxidant gene (Guerrero-Beltrán et al. 2012). This protects the testis from oxidative stress, preventing testicular inflammation and cell death. For diabetic-treated rats, SFN-treated diabetic mellitus (DM) mice exhibited higher testicular Nrf2 expression and function (Guerrero-Beltrán et al. 2012; Wang et al. 2014). SFN also enhanced Nrf2 mRNA and protein expression in rats after cadmium exposure. SFN reduces cadmium-induced reproductive damage by activating the mRNA expression of Nrf2 and modulating phase 2 antioxidant enzymes and detoxification (Qin et al. 2017; Yang et al. 2016, 2018).

Squid ink polysaccharides (SIP) are glycosaminoglycans with antioxidative and chemoprotective activities (Le et al. 2015). SIP decreased the content of KEAP1 protein, which binds and also increases dissociative NRF2 protein in the cytoplasm and combined NRF2 protein bound to AREs in the nucleus (Le et al. 2015). The findings showed that SIP not only decreased KEAP1 gene expression in normal mice's testes but also favorably influenced protein content, which was subjected to resisting the increase in Nrf2 gene expression. To protect mice testis from CP-induced damage, SIP used the Nrf2/ARE signal pathway to activate downstream target genes HO1 and NQO1, whereas protein kinase C (PKC) acted as an upstream regulator in the Nrf2 regulatory process (Le et al. 2015).

Table 1 The activation of Nrf2 in the testis

S/No	Activator	Mechanism	Ref.
1	**MitoQ**	Enhanced Nrf2 expression Decreased the KEAP1 expression Promoted Nrf2 nuclear translocation	Xiao et al. (2017)
2	**Sulforaphane (SFN)**	Increased the expression of Nrf2 and downstream antioxidant gene Protects cells against oxidative damage Activate Nrf2/ARE pathways	Guerrero-Beltrán et al. (2012); Wang et al. (2014)
3	**Squid ink polysaccharides (SIP)**	Enhanced Nrf2 expression Decrease KEAP1 gene expression Activate downstream target genes HO1 and NQO1	Le et al. (2015)
4	**Umbelliferone (UMB)**	Reducing oxidative damage Activating Nrf2/HO-1 signaling	Alotaibi et al. (2020)
5	**Tadalafil (TDF)**	Enhanced Nrf2 and HO-1 expression	Abdel-Wahab et al. (2020)
6	**GSPE (grape seed proanthocyanidin extract)**	Increased the expression of Nrf2 and HO 1 Activates Nrf2 signaling pathway	Wang et al. (2018)
7	**MOTILIPERM extract**	Increased the Nrf2 and HO-1 levels Increased antioxidant levels	Karna et al. (2020)
8	**Aucubin (AU)**	Suppress JNK and CHOP activation Triggered nuclear translocation of Nrf2 for protection	Ma et al. (2019a)
9	**Proanthocyanidins (PAs)**	Increased expression of Nrf2 and target proteins decreased the expression of KEAP1	He et al. (2018)
10	**N-acetylcysteine (NAC)**	Enhanced Nrf2 expression	Jannatifar et al. (2020)
11	**Zinc (Zn)**	Enhanced Nrf2 expression and its downstream antioxidant gene	Maremanda et al. (2014), Maremanda et al. (2016)
12	**Melatonin**	Increased the expression of Nrf2 and HO 1	Guo et al. (2017)
13	**Luteolin**	Nrf2 activation Elevated HO-1 and NQO1 gene and protein expression	Ma et al. (2019b)
14	**Lutein (LU)**	Enhanced Nrf2 expression and its downstream antioxidant gene	Li et al. (2016)
15	**Lycopene (LYC)**	Increased mRNA expression of Nrf2 and its downstream antioxidant genes	Zhao et al. (2019)
16	**Paeonol**	Reduces testicular lipid peroxidation Restoring GSH levels and SOD activity Activation of Nrf2 expression	Li et al. (2019)
17	**Soy isoflavones**	Increased the expression of Nrf2 and HO 1	Luo et al. (2019)

Umbelliferone (UMB) is a coumarin with protective activities against cytotoxicity. The Pb-induced testicular injury was prevented by UMB by reducing oxidative damage and increasing antioxidant defenses, as well as Nrf2/HO-1 signaling (Alotaibi et al. 2020).

Tadalafil (TDF) is used to treat erectile dysfunction as it inhibits phosphodiesterase-5 (PDE5). According to Abdel-Wahab et al. (2020), the protective effect of TDF against cisplatin-induced testicular damage may be achieved through Nrf2/HO-1 signaling pathway activation. According to their findings, TDF reduced oxidative stress and tissue damage in cisplatin-induced testicular injury by increasing the activities of HO-1 and Nrf-2.

GSPE (grape seed proanthocyanidin extract) is a polyphenolic bioflavonoid with a wide range of biological, pharmacological, and therapeutic activities in defense against oxidative stress (Eugenio-Pérez et al. 2016). In several studies, GSPE has been shown to activate the Nrf2 pathway through its antioxidative effect (Chen et al. 2015; Rajput et al. 2019; Sun et al. 2016). However, Wang et al. (2018) focused on the relationship between GSPE and the Nrf2 signaling pathway in the testis, revealing that GSPE significantly increased the expression of Nrf2 and HO-1.

Motiliperm extract is composed of three crude medicinal herbs: the root of *Morinda officinalis* (Rubiaceae), the outer scales of *Allium cepa* L. (Liliaceae), and the seeds

of *Cuscuta chinensis* Lamark (convolvulaceae) (Soni et al. 2018). The study by Karna et al. (2020) demonstrated an antioxidant response mediated by the Nrf2/HO-1 pathway in stressed rats after MOTILIPERM therapy. In stressed rats, MOTILIPERM therapy increased the levels of Nrf2, catalase, SOD, and HO-1. MOTILIPERM stimulates the Nrf2/HO-1 signaling pathway through antioxidant and anti-apoptotic actions.

Aucubin (AU) is a natural iridoid glucoside derived from the leaves of *Eucommia ulmoides* that has a high antioxidant and anti-inflammatory effect in the treatment of a variety of illnesses (Wang et al. 2015). AU reduces oxidative stress-induced testis damage by suppressing JNK and CHOP activation via Nrf2 upregulation (Chun Li et al. 2021, Ma et al. 2019a). Nrf2 activity might be the target of AU's preventive effect against testicular damage caused by oxidative stress. According to Ma et al. (2020), AU triggers nuclear translocation of Nrf2 and consistently protects against triptolide-induced decreases in antioxidant enzyme expression.

Proanthocyanidins (PAs) are flavan-3-ols found in plants that are naturally occurring (Liu et al. 2016). Perhaps due to a change in the Nrf2-KEAP1 signaling pathway, PAs had a considerable chemo-preventive effect in rats against Cd-induced testicular oxidative damage. PAs increased the expression of Nrf2 and its target proteins, but they decreased the expression of KEAP1 (He et al. 2018).

N-acetylcysteine (NAC) is an important scavenger of hydroxyl radicals that regulates intracellular redox effectors involved in testicular morphology and semen quality (Feng et al. 2015). NAC can cause Nrf2 to translocate to the nucleus, resulting in the activation of the Nrf2/ARE-mediated antioxidant response (Jannatifar et al. 2020). NAC therapy resulted in a partial reversal of the low level of NRF2 protein in the cytosol caused by sodium fluoride (NaF), showing that NAC treatment was successful in sequestering testicular Nrf2 into the nucleus in response to NaF (Hu et al. 2019).

Zinc (Zn) is an essential trace element in the body, as it is a component of over 300 enzymes that are necessary for cell homeostasis, growth, and development (Khan and Awan 2014). The level of Nrf2, as well as downstream pathway molecules, was considerably restored by Zn. Zn, which is also a component of the antioxidant system, has been shown to influence Nrf2 expression (Maremanda et al. 2014, 2016).

Melatonin is an antioxidant produced mainly by the pineal gland and may scavenge different free radicals (Xu et al. 2013). Melatonin also protects numerous tissues from oxidant-induced damage by upregulating the production of antioxidant proteins (Yang et al. 2019a).. The capacity of melatonin to control the Nrf2 pathway has also been linked to the regulation of HO-1 expression. Guo et al. (2017) observed that melatonin significantly increased both Nrf2 and HO-1 in stress-treated testes and H_2O_2-treated testicular cells. However, it is unclear whether HO-1 is directly controlled by melatonin or is dependent on Nrf2.

Luteolin possesses potent antioxidative and cytoprotective activities, which may be due to its regulation of the Nrf2 pathway (Chian et al. 2014). In Sertoli cells, Ma et al. (2020) found that luteolin treatment increased the Nrf2 accumulation in the nuclear fraction and induced Nrf2 transactivation activity, resulting in reduced oxidative damage and apoptosis. Furthermore, in vivo studies indicated comparable effects, including Nrf2 activation and significantly elevated HO-1 and NQO1 gene and protein expression in testis tissue (Liu et al. 2016).

Lutein (LU) is xanthophyll made only by plants and is found in abundance in green leafy vegetables like spinach, yellow carrots, and kale, as well as lettuce and broccoli (Manayi et al. 2016). Li et al. (2016) found that LU activated Nrf2 to protect against arsenic-induced oxidative damage; the expression of Nrf2 levels and its downstream genes improved after administration with LU.

Lycopene (LYC) is a carotenoid found mostly in red fruits and vegetables such as watermelon, tomatoes, and papayas. Because of its many therapeutic benefits, including enhancing reproductive function (Sumathy 2016; Zhao et al. 2020), LYC has received much interest. LYC could reduce oxidative stress by increasing antioxidative gene levels and Nrf2 nuclear translocation. Zhao et al. (2019) found that LYC increased the mRNA expression of Nrf2 and its downstream antioxidant genes, with a trend that is identical to their protein expression. Thus, LYC counteracts the generated oxidative stress in Leydig cells by altering Nrf2 activity and transcription.

Paeonol (2-hydroxy-4-methoxyacetophenone) is a phenolic compound with anti-inflammatory and antioxidant activities (Zong et al. 2018). According to Li et al. (2019), paeonol could alleviate oxidative stress in ischemic reperfusion (IR) rats by reducing testicular lipid peroxidation and restoring GSH levels and SOD activity as a result of activation of Nrf2 expression. Also, Mohamed et al. (2020) revealed that paeonol-treated rats exhibited a substantial rise in testicular Nrf2 levels.

Soy isoflavones (SIF) are abundantly found in soybeans and are also bioactive compounds with non-steroidal and phenolic properties with strong antioxidant activity. SIF treatment has been found to stimulate Nrf2-mediated antioxidant responses in the testis (Yalcin and Çapar 2017). Nrf2 activates HO-1, an enzyme that catalyzes the conversion of heme to carbon monoxide (CO) and free iron (Fe), as well as the conversion of biliverdin to bilirubin. Both CO and bilirubin have antioxidant properties. Increasing the expression of HO-1 protects against testicular oxidative stress (Luo et al. 2019).

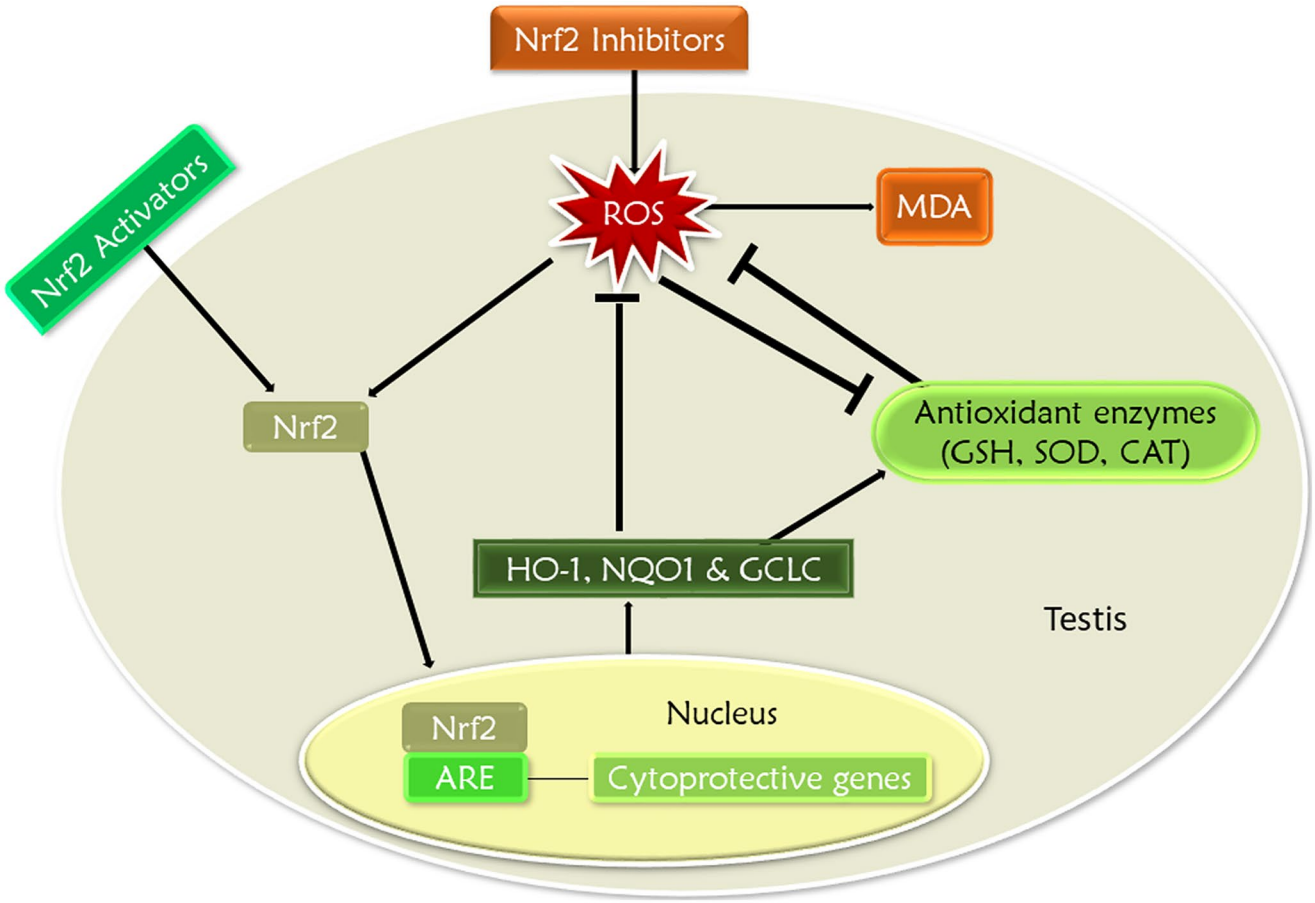

Fig. 3 The mechanism of activation and inhibition of Nrf2 in the testis

Inhibition of Nrf2 Several factors and compounds have been reported to inhibit Nrf2. Nrf2 inhibitors facilitate oxidative stress via interfering with the Nrf2 signaling pathway (Table 1; Fig. 3).

Heat stress The formation of reactive oxygen species (ROS) is usually induced by heat stress in cells (Belhadj Slimen et al. 2014). Scrotal heat regulated the Nrf2 production and translocation of NRF2 protein into Leydig cells, as well as higher mRNA levels for Nrf2-regulated genes (Li et al. 2013). Also, concentrated NRF2 protein was observed in the cytoplasm of elongated spermatids (Li et al. 2013).

Diabetes Diabetes mellitus (DM) is a group of multi-systemic disorders that threaten several organs, including the testis. Ebokaiwe et al. (2020) observed a significant reduction in testicular Nrf2 expression in diabetic untreated rats, indicating a severe oxidative stress response to hyperglycemia. Diabetes could also suppress Nrf2 and promote testicular apoptosis in type 1 diabetes, according to Jiang et al. (2014). Similarly, Wang et al. (2014), Maremanda et al. [65], and Zhao et al. (2020) found that HO-1 and NQO-1 expression had decreased considerably in the testis of T2DM mice. These results suggest that diabetes inhibits not only the expression of Nrf2 but also its function.

DEHP Di-(2-ethylhexyl) phthalate is widely used as a plasticizer and also an environmental endocrine disruptor that is hazardous to the male reproductive system and is widely used in China (Liu et al. 2014). The protective effect of Nrf2 is decreased in response to excessive exposure to toxins like DEHP (Zhao et al. 2019). Studies have shown that DEHP exposure interferes with the Nrf2 signal pathway and facilitated oxidative stress (Amara et al. 2020; Tang et al. 2018). DEHP caused testicular damage by oxidative stress, which decreased the expression of Nrf2.

Cadmium (Cd) It is a heavy metal with a potential environmental hazard. Cd treatment reduces Nrf2 and regulates the expression of NQO1, HO1, GCS, and GPx in the testis while increasing KEAP1 expression levels (Shi and Fu 2019). The findings were consistent with those of He et al. (2018) and Yang et al. (2018) who reported that cadmium-induced oxidative stress considerably enhances gene transcription. This

Table 2 The inhibition of Nrf2 in the testis

S/No	Inhibitor	Mechanism	Ref.
1	***Heat stress***	Reduced Nrf2 production	Li et al. (2013)
2	***Diabetes***	Reduction in testicular Nrf2 expression Promotes testicular apoptosis Decreased HO-1 and NQO-1 expression	Wang et al. (2014)
3	**Di-(2-ethylhexyl) phthalate *(DEHP)***	Interfere with the Nrf2 signal pathway	Tang et al. (2018)
4	***Cadmium* (Cd)**	Reduces Nrf2 expressions Suppress the expressions of NQO1, HO1 & GCS Increasing KEAP1 expression	He et al. (2018)
5	***Fluoride***	Reduces the expression of Nrf2 and its target genes	Hu et al. (2019)
6	***Anticancer drugs***	Cyclophosphamide reduced Nrf2 levels and Nrf2 downstream pathway genes Doxorubicin deactivate Nrf2 pathway via inhibition of antioxidant enzymes and increased oxidative stress	Maremanda et al. (2016), Renu and Gopalakrishnan (2019)
7	***Arsenic***	Reduced expression of Nrf2 and its downstream genes	Li et al. (2016)
8	***Cyclosporine***	Reduced Nrf2/HO-1 level	Kabel et al. (2020)
9	***4-Nitrophenol (PNP)***	Reduces the expression of Nrf2 and its target genes	Mi et al. (2013)

demonstrated that exogenous Cd may inhibit the inherent antioxidant capacity in the testicular cells by decreasing the production of Nrf2 and its regulated genes, resulting in oxidant stress (Fig. 3).

Fluoride The formation of oxidative stress is the primary focus for mediating reproductive damage caused by excess fluorides, such as sperm production impairment. After NaF exposure, Hu et al. (2019) observed that the mRNA expression of the Nrf2 target genes HO-1 and NQO1 decreased considerably. Interestingly, the protein expression of these genes displayed the same modification. Hu et al. (2019) also revealed that the mRNA expression of Nrf2 target genes, NQO1, and HO-1 showed a slight downregulation after NaF exposure (Fig. 3).

Anticancer drugs One of the most widely used anticancer drugs, cyclophosphamide (CP), has been used for many decades. According to Maremanda et al. (2014), CP reduced Nrf2 levels in the testes. Furthermore, CP treatment disrupted Nrf2 downstream pathway genes (HO-1 and NQO1). Also, Renu and Gopalakrishnan (2019) reported that the action of doxorubicin showed altered redox status due to the deactivation of the Nrf2 pathway via inhibition of antioxidant enzymes and increased oxidative stress (LPO and ROS) markers. Cisplatin (CIS) is an essential anticancer drug that is used to treat a variety of cancers (Mercantepe et al. 2018). Abdel-Wahab et al. (2020) observed a decrease in HO-1 activity in cisplatin-treated rats, which could be a result of the stressed condition created by the toxic effects of CIS in the testis. The toxicity caused by CIS produces a large amount of ROS and RNS, which exceeds the cellular antioxidant enzymes' presence. Thus, the activities of the antioxidant enzymes become depleted and unable to combat the ensuing testicular damage (Fig. 3).

Arsenic Arsenic poisoning is a medical condition that occurs as a result of high arsenic levels in the body (Sinha et al. 2013). Antioxidant enzymes are affected by arsenic. The levels of mRNA expression and protein expression of Nrf2 and its downstream genes were reduced after the administration of arsenic (Li et al. 2016). These findings suggest that arsenic inhibits Nrf2 signaling, leading to an increase in the oxidative stress marker and reducing the antioxidant marker in the cell (Fig. 3).

Cyclosporine It is an immunosuppressive drug used to minimize organ rejection following organ transplants. Kabel et al. (2020) indicated the importance of the Nrf2 pathway in cyclosporine-induced testicular toxicity; the administration of cyclosporine stimulated a marked decline in the Nrf2/HO-1 level. Also, Arab et al. (2021) reported that the Nrf2/HO-1 signaling pathway regulates the toxic effects of cyclosporine A in the rat testis.

4-Nitrophenol (PNP) It is essential in the production of organic compounds. It is used for engineering polymers, dyes, and pigment production; in agriculture as a pesticide, pharmaceutical, and organic synthesis; as a fungicide for leather; and also for military applications (Arora et al. 2014; Mi et al. 2013). Previous studies have shown that PNP inhibits the testicular expression of hormone receptors, reduces serum hormonal balance, triggers oxidative stress, and inhibits the Nrf2 signaling pathway, along with the accumulation of Nrf2 in the nuclei of Leydig cells and germ cells (Yang et al. 2019a; Zhang et al. 2016, 2013). The regulation might

be connected to the modification of mRNA expression of Nrf2, GCLC, and HO-1 due to oxidative stress (Fig. 3 and Table 2).

Conclusions and future prospects

The signaling pathway of Nrf2 is regarded as essential in maintaining the redox status within the cell. This review described the effects of Nrf2 activators and inhibitors and their role in targeting oxidative stress as a therapeutic option. In the testis, Nrf2 inhibitors could facilitate oxidative stress, producing adverse effects that could result in impairment of the Nrf2 signaling pathway and testicular dysfunction. Considering this fact, Nrf2 may be a potential therapeutic candidate to combat testicular oxidative stress and improve antioxidant balance. Nevertheless, there could be some challenges that may limit the use of this therapeutic strategy. Low bioavailability of compounds (Nrf2 activator) due to the blood-testis barrier could hinder the therapeutic prospects. However, nanocarriers enhance the bioavailability of therapeutic agents and demonstrate great potential in crossing over the blood-testis barrier.

The Nrf2 signaling pathway is a novel therapeutic target in the testis, and future studies are needed to clarify the role of this Nrf2 pathway in inhibiting testicular oxidative stress. Also, clinical trials on the effects of the Nrf2 signaling pathway in human testes have been poorly investigated. Future research needs to look into the dosage and length of treatment to establish the role of Nrf2 activation.

Author contribution RDE conceived and designed the study; RDE, OTD, OAO, and AOS collaborated in the discussion of the results. All authors wrote the manuscript and provided administrative support and critically revised the manuscript. All the authors have and approved the final manuscript.

Data availability Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Abdel-Wahab BA, Alkahtani SA, Elagab EA (2020) Tadalafil alleviates cisplatin-induced reproductive toxicity through the activation of the Nrf2/HO-1 pathway and the inhibition of oxidative stress and apoptosis in male rats. Reprod Toxicol 96:165–174

Alotaibi MF, Al-Joufi F, Abou Seif HS, Alzoghaibi MA, Djouhri L, Ahmeda AF, Mahmoud AM (2020) Umbelliferone inhibits spermatogenic defects and testicular injury in lead-intoxicated rats by suppressing oxidative stress and inflammation, and improving Nrf2/HO-1 signaling. Drug Des Dev Ther 14:4003

Amara I, Salah A, Timoumi R, Annabi E, Scuto M, Trovato A, Neffati F, Calabrese V, Abid-Essefi S (2020) Effect of di (2-ethylhexyl) phthalate on Nrf2-regulated glutathione homeostasis in mouse kidney. Cell Stress Chaperones 25:919–928

Arab HH, Ashour AM, Alqarni AM, Arafa E-SA, Kabel AM (2021) Camel milk mitigates cyclosporine-induced renal damage in rats: targeting p38/ERK/JNK MAPKs, NF-κB, and matrix metalloproteinases. Biology 10:442

Arora PK, Srivastava A, Singh VP (2014) Bacterial degradation of nitrophenols and their derivatives. J Hazard Mater 266:42–59

Belhadj Slimen I, Najar T, Ghram A, Dabbebi H, Ben Mrad M, Abdrabbah M (2014) Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A Review International Journal of Hyperthermia 30:513–523

Bhakkiyalakshmi E, Sireesh D, Rajaguru P, Paulmurugan R, Ramkumar KM (2015) The emerging role of redox-sensitive Nrf2–Keap1 pathway in diabetes. Pharmacol Res 91:104–114

Bieniek JM, Drabovich AP, Lo KC (2016) Seminal biomarkers for the evaluation of male infertility. Asian J Androl 18:426

Bryan HK, Olayanju A, Goldring CE, Park BK (2013) The Nrf2 cell defence pathway: Keap1-dependent and-independent mechanisms of regulation. Biochem Pharmacol 85:705–717

Chen S, Zhu Y, Liu Z, Gao Z, Li B, Zhang D, Zhang Z, Jiang X, Liu Z, Meng L (2015) Grape seed proanthocyanidin extract ameliorates diabetic bladder dysfunction via the activation of the Nrf2 pathway. PLoS ONE 10:e0126457

Chian S, Thapa R, Chi Z, Wang XJ, Tang X (2014) Luteolin inhibits the Nrf2 signaling pathway and tumor growth in vivo. Biochem Biophys Res Commun 447:602–608

Chun Li Y, Cheng Hao J, Shang B, Zhao C, Juan Wang L, lin Yang K, Zhou He X, Gian Tian Q, Liang Wang Z, Ling Jing H (2021) Neuroprotective effects of aucubin on hydrogen peroxide-induced toxicity in human neuroblastoma SH-SY5Y cells via the Nrf2/HO-1 pathway. Phytomedicine 87:153577

Cuadrado A, Rojo AI, Wells G, Hayes JD, Cousin SP, Rumsey WL, Attucks OC, Franklin S, Levonen A-L, Kensler TW (2019) Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat Rev Drug Discov 18:295–317

Dutta S, Majzoub A, Agarwal A (2019) Oxidative stress and sperm function: a systematic review on evaluation and management. Arab J Urol 17:87–97

Ebokaiwe AP, Osawe S, Griffin S, Keck CM, Olusanya O, Ehiri RC (2020) Loranthus micranthus nanoparticles abates streptozotocin-instigated testicular dysfunction in Wistar rats: involvement of glucose metabolism enzymes, oxido-inflammatory stress, steroidogenic enzymes/protein and Nrf2 pathway. Andrologia 52:e13749

Eugenio-Pérez D, Medina-Fernández LY, Saldivar-Anaya JA, Molina-Jijón E, Pedraza-Chaverri J (2016) Role of dietary antioxidant agents in chronic kidney. Free Radicals Dis 1:331

Feng D, Huang H, Yang Y, Yan T, Jin Y, Cheng X, Cui L (2015) Ameliorative effects of N-acetylcysteine on fluoride-induced oxidative stress and DNA damage in male rats' testis. Mutat Res/gen Toxicol Environ Mutagen 792:35–45

Guerrero-Beltrán CE, Calderón-Oliver M, Pedraza-Chaverri J, Chirino YI (2012) Protective effect of sulforaphane against oxidative stress: recent advances. Exp Toxicol Pathol 64:503–508

Guo Y, Sun J, Li T, Zhang Q, Bu S, Wang Q, Lai D (2017) Melatonin ameliorates restraint stress-induced oxidative stress and apoptosis in testicular cells via NF-κB/iNOS and Nrf2/HO-1 signaling pathway. Sci Rep 7:1–13

Hayes JD, Dinkova-Kostova AT (2014) The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci 39:199–218

He L, Li P, Yu L-H, Li L, Zhang Y, Guo Y, Long M, He J-B, Yang S-H (2018) Protective effects of proanthocyanidins against cadmium-induced testicular injury through the modification of Nrf2-Keap1 signal path in rats. Environ Toxicol Pharmacol 57:1–8

Hu Y, Wang Y, Yan T, Feng D, Ba Y, Zhang H, Zhu J, Cheng X, Cui L, Huang H (2019) N-acetylcysteine alleviates fluoride-induced testicular apoptosis by modulating IRE1α/JNK signaling and nuclear Nrf2 activation. Reprod Toxicol 84:98–107

Ishitsuka Y, Ogawa T, Roop D (2020) The KEAP1/NRF2 signaling pathway in keratinization. Antioxidants 9:751

Jannatifar R, Parivar K, Roodbari NH, Nasr-Esfahani MH (2020) The effect of N-acetyl-cysteine on NRF2 antioxidant gene expression in asthenoteratozoospermia men: a clinical trial study. Int J Fertil Steril 14:171

Jiang X, Bai Y, Zhang Z, Xin Y, Cai L (2014) Protection by sulforaphane from type 1 diabetes-induced testicular apoptosis is associated with the up-regulation of Nrf2 expression and function. Toxicol Appl Pharmacol 279:198–210

Kabel AM, Salama SA, Alghorabi AA, Estfanous RS (2020) Amelioration of cyclosporine-induced testicular toxicity by carvedilol and/or alpha-lipoic acid: role of TGF-β1, the proinflammatory cytokines, Nrf2/HO-1 pathway and apoptosis. Clin Exp Pharmacol Physiol 47:1169–1181

Karna KK, Soni KK, You JH, Choi NY, Kim HK, Kim CY, Lee SW, Shin YS, Park JK (2020) MOTILIPERM ameliorates immobilization stress-induced testicular dysfunction via inhibition of oxidative stress and modulation of the Nrf2/HO-1 pathway in SD rats. Int J Mol Sci 21:4750

Kayode OT, Rotimi DE, Olaolu TD, Adeyemi OS (2020) Ketogenic diet improves and restores redox status and biochemical indices in monosodium glutamate-induced rat testicular toxicity. Biomed Pharmacother 127:110227

Keum Y-S, Choi BY (2014) Molecular and chemical regulation of the Keap1-Nrf2 signaling pathway. Molecules 19:10074–10089

Khan AR, Awan FR (2014) Metals in the pathogenesis of type 2 diabetes. J Diabetes Metab Disord 13:1–6

Kitamura H, Motohashi H (2018) NRF2 addiction in cancer cells. Cancer Sci 109:900–911

Krajka-Kuźniak V, Paluszczak J, Baer-Dubowska W (2017) The Nrf2-ARE signaling pathway: an update on its regulation and possible role in cancer prevention and treatment. Pharmacol Rep 69:393–402

Kumar H, Kim I-S, More SV, Kim B-W, Choi D-K (2014) Natural product-derived pharmacological modulators of Nrf2/ARE pathway for chronic diseases. Nat Prod Rep 31:109–139

Le X, Luo P, Gu Y, Tao Y, Liu H (2015) Interventional effects of squid ink polysaccharides on cyclophosphamide-associated testicular damage in mice. Bratisl Lek Listy 116:334–339

Li J, Li Y, Pan S, Zhang L, He L, Niu Y (2019) Paeonol attenuates ligation-induced periodontitis in rats by inhibiting osteoclastogenesis via regulating Nrf2/NF-κB/NFATc1 signaling pathway. Biochimie 156:129–137

Li S, Xu S, Niu Q, Ding Y, Pang L, Ma R, Jing M, Wang K, Ma X, Feng G (2016) Lutein alleviates arsenic-induced reproductive toxicity in male mice via Nrf2 signaling. Hum Exp Toxicol 35:491–500

Li Y, Cao Y, Wang F, Li C (2014) Scrotal heat induced the Nrf2-driven antioxidant response during oxidative stress and apoptosis in the mouse testis. Acta Histochem 116:883–890

Li Y, Huang Y, Piao Y, Nagaoka K, Watanabe G, Taya K, Li C (2013) Protective effects of nuclear factor erythroid 2-related factor 2 on whole body heat stress-induced oxidative damage in the mouse testis. Reprod Biol Endocrinol 11:1–10

Liu C, Wang X, Shulaev V, Dixon RA (2016) A role for leucoanthocyanidin reductase in the extension of proanthocyanidins. Nature Plants 2:1–7

Liu T, Li N, Zhu J, Yu G, Guo K, Zhou L, Zheng D, Qu X, Huang J, Chen X (2014) Effects of di-(2-ethylhexyl) phthalate on the hypothalamus-pituitary-ovarian axis in adult female rats. Reprod Toxicol 46:141–147

Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J (2016) Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci 73:3221–3247

Luo Q, Li Y, Huang C, Cheng D, Ma W, Xia Y, Liu W, Chen Z (2019) Soy isoflavones improve the spermatogenic defects in diet-induced obesity rats through Nrf2/HO-1 pathway. Molecules 24:2966

Ma B, Zhang J, Zhu Z, Bao X, Zhang M, Ren C, Zhang Q (2019a) Aucubin, a natural iridoid glucoside, attenuates oxidative stress-induced testis injury by inhibiting JNK and CHOP activation via Nrf2 up-regulation. Phytomedicine 64:153057

Ma B, Zhang J, Zhu Z, Zhao A, Zhou Y, Ying H, Zhang Q (2019b) Luteolin ameliorates testis injury and blood–testis barrier disruption through the Nrf2 signaling pathway and by upregulating Cx43. Mol Nutr Food Res 63:1800843

Ma B, Zhu Z, Zhang J, Ren C, Zhang Q (2020) Aucubin alleviates diabetic nephropathy by inhibiting NF-κB activation and inducing SIRT1/SIRT3-FOXO3a signaling pathway in high-fat diet/streptozotocin-induced diabetic mice. J Function Foods 64:103702

Manayi A, Abdollahi M, Raman T, Nabavi SF, Habtemariam S, Daglia M, Nabavi SM (2016) Lutein and cataract: from bench to bedside. Crit Rev Biotechnol 36:829–839

Maremanda KP, Khan S, Jena G (2014) Zinc protects cyclophosphamide-induced testicular damage in rat: involvement of metallothionein, tesmin and Nrf2. Biochem Biophys Res Commun 445:591–596

Maremanda KP, Khan S, Jena G (2016) Role of zinc supplementation in testicular and epididymal damages in diabetic rat: involvement of Nrf2, SOD1, and GPX5. Biol Trace Elem Res 173:452–464

Mercantepe T, Unal D, Tümkaya L, Yazici ZA (2018) Protective effects of amifostine, curcumin and caffeic acid phenethyl ester against cisplatin-induced testis tissue damage in rats. Exp Ther Med 15:3404–3412

Mi Y, Tu L, Wang H, Zeng W, Zhang C (2013) Supplementation with quercetin attenuates 4-nitrophenol-induced testicular toxicity in adult male mice. Anat Rec 296:1650–1657

Mohamed MZ, Morsy MA, Mohamed HH, Hafez HM (2020) Paeonol protects against testicular ischaemia-reperfusion injury in rats through inhibition of oxidative stress and inflammation. Andrologia 52:e13599

Moon EJ, Giaccia A (2015) Dual roles of NRF2 in tumor prevention and progression: possible implications in cancer treatment. Free Radical Biol Med 79:292–299

Moreno-Fernandez J, Alférez MJ, López-Aliaga I, Diaz-Castro J (2019) Protective effects of fermented goat milk on genomic stability, oxidative stress and inflammatory signalling in testis during anaemia recovery. Sci Rep 9:1–11

Ojo OA, Ajiboye B, Fadaka A, Taro P, Shariati MA (2017) Nrf2-Keap1 activation, a promising strategy in the prevention of cancer. Free Radacals Antioxid 7:1–7

Olaolu D, Rotimi DE, Olaolu AP (2018) Effect of alcohol infusion of Cissus populnea root on testicular function and serum hormone of male Wistar rats. Asian Pacific J Reproduct 7:117–122

Pallesen JS, Tran KT, Bach A (2018) Non-covalent small-molecule Kelch-like ECH-associated protein 1–nuclear factor erythroid 2-related factor 2 (Keap1–Nrf2) inhibitors and their potential for targeting central nervous system diseases. J Med Chem 61:8088–8103

Qin Z, Tang J, Han P, Jiang X, Yang C, Li R, Tang M, Shen B, Wang W, Qin C (2017) Protective effects of sulforaphane on di-n-butylphthalate-induced testicular oxidative stress injury in male mice offsprings via activating Nrf2/ARE pathway. Oncotarget 8:82956

Rajput SA, Sun L, Zhang N-Y, Khalil MM, Ling Z, Chong L, Wang S, Rajput IR, Bloch DM, Khan FA (2019) Grape seed proanthocyanidin extract alleviates aflatoxinB1-induced immunotoxicity and oxidative stress via modulation of NF-κB and Nrf2 signaling pathways in broilers. Toxins 11:23

Renu K, Gopalakrishnan AV (2019) Deciphering the molecular mechanism during doxorubicin-mediated oxidative stress, apoptosis through Nrf2 and PGC-1α in a rat testicular milieu. Reprod Biol 19:22–37

Rössler OG, Thiel G (2017) Specificity of stress-responsive transcription factors Nrf2, ATF4, and AP-1. J Cell Biochem 118:127–140

Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR (2016) Etiologies of sperm oxidative stress. Int J Reproduct Biomed 14:231

Saha S, Buttari B, Panieri E, Profumo E, Saso L (2020) An overview of Nrf2 signaling pathway and its role in inflammation. Molecules 25:5474

Sengupta P, Nwagha U, Dutta S, Krajewska-Kulak E, Izuka E (2017) Evidence for decreasing sperm count in African population from 1965 to 2015. Afr Health Sci 17:418–427

Shahat A, Rizzoto G, Kastelic J (2020) Amelioration of heat stress-induced damage to testes and sperm quality. Theriogenology 158:84–96

Shi X, Fu L (2019) Piceatannol inhibits oxidative stress through modification of Nrf2-signaling pathway in testes and attenuates spermatogenesis and steroidogenesis in rats exposed to cadmium during adulthood. Drug Des Dev Ther 13:2811

Sihvola V, Levonen A-L (2017) Keap1 as the redox sensor of the antioxidant response. Arch Biochem Biophys 617:94–100

Sinha D, Biswas J, Bishayee A (2013) Nrf2-mediated redox signaling in arsenic carcinogenesis: a review. Arch Toxicol 87:383–396

Soni KK, Zhang LT, Choi BR, Karna KK, You JH, Shin YS, Lee SW, Kim CY, Zhao C, Chae H-J (2018) Protective effect of MOTILIPERM in varicocele-induced oxidative injury in rat testis by activating phosphorylated inositol requiring kinase 1α (p-IRE1α) and phosphorylated c-Jun N-terminal kinase (p-JNK) pathways. Pharm Biol 56:94–103

Sumathy VJH (2016) Identification of Lyc Studies. Int J Curnt Tren Pharm Res 4:351–354

Sun C, Liu X, Di C, Wang Z, Mi X, Liu Y, Zhao Q, Mao A, Chen W, Gan L (2017) MitoQ regulates autophagy by inducing a pseudo-mitochondrial membrane potential. Autophagy 13:730–738

Sun Y, Xiu C, Liu W, Tao Y, Wang J, Qu Y (2016) Grape seed proanthocyanidin extract protects the retina against early diabetic injury by activating the Nrf2 pathway. Exp Ther Med 11:1253–1258

Tang X, Wu S, Shen L, Wei Y, Cao X, Wang Y, Long C, Zhou Y, Li D, Huang F (2018) Di-(2-ethylhexyl) phthalate (DEHP)-induced testicular toxicity through Nrf2-mediated Notch1 signaling pathway in Sprague-Dawley rats. Environ Toxicol 33:720–728

Tonelli C, Chio IIC, Tuveson DA (2018) Transcriptional regulation by Nrf2. Antioxid Redox Signal 29:1727–1745

Tong Y-H, Zhang B, Fan Y, Lin N-M (2015) Keap1-Nrf2 pathway: a promising target towards lung cancer prevention and therapeutics. Chronic Dis Translation Med 1:175–186

Uddin MS, Al Mamun A, Jakaria M, Thangapandiyan S, Ahmad J, Rahman MA, Mathew B, Abdel-Daim MM, Aleya L (2020) Emerging promise of sulforaphane-mediated Nrf2 signaling cascade against neurological disorders. Sci Total Environ 707:135624

Wajda A, Łapczuk J, Grabowska M, Słojewski M, Laszczyńska M, Urasińska E, Droździk M (2016) Nuclear factor E2-related factor-2 (Nrf2) expression and regulation in male reproductive tract. Pharmacol Rep 68:101–108

Wang S-n, Xie G-p, Qin C-h, Chen Y-r, Zhang K-r, Li X, Wu Q, Dong W-q, Yang J, Yu B (2015) Aucubin prevents interleukin-1 beta induced inflammation and cartilage matrix degradation via inhibition of NF-κB signaling pathway in rat articular chondrocytes. Int Immunopharmacol 24:408–415

Wang Y, Chen F, Liang M, Chen S, Zhu Y, Zou Z, Shi B (2018) Grape seed proanthocyanidin extract attenuates varicocele-induced testicular oxidative injury in rats by activating the Nrf2-antioxidant system. Mol Med Rep 17:1799–1806

Wang Y, Zhang Z, Guo W, Sun W, Miao X, Wu H, Cong X, Wintergerst KA, Kong X, Cai L (2014) Sulforaphane reduction of testicular apoptotic cell death in diabetic mice is associated with the upregulation of Nrf2 expression and function. Am J Physiol-Endocrinol Metab 307:E14–E23

Wu S, Lu H, Bai Y (2019) Nrf2 in cancers: a double-edged sword. Cancer Med 8:2252–2267

Xiao L, Xu X, Zhang F, Wang M, Xu Y, Tang D, Wang J, Qin Y, Liu Y, Tang C (2017) The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. Redox Biol 11:297–311

Xu C, Wu A, Zhu H, Fang H, Xu L, Ye J, Shen J (2013) Melatonin is involved in the apoptosis and necrosis of pancreatic cancer cell line SW-1990 via modulating of Bcl-2/Bax balance. Biomed Pharmacother 67:133–139

Yalcin H, Çapar TD (2017) Bioactive compounds of fruits and vegetables. Minimally Processed Refrigerated Fruits and Vegetables. Springer, pp 723–745

Yamamoto M, Kensler TW, Motohashi H (2018) The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol Rev 98:1169–1203

Yang ZhuJ, Luo X, Li F, Cong L, Wang Y, Sun Y (2019a) Melatonin attenuates cadmium-induced ovulatory dysfunction by suppressing endoplasmic reticulum stress and cell apoptosis. Reprod Biol Endocrinol 17:1–8

Yang L, Palliyaguru DL, Kensler TW (2016) Frugal chemoprevention: targeting Nrf2 with foods rich in sulforaphane. Sem Oncol 43. Elsevier, pp 146–153

Yang S-H, He J-B, Yu L-H, Li L, Long M, Liu M-D, Li P (2019b) Protective role of curcumin in cadmium-induced testicular injury in mice by attenuating oxidative stress via Nrf2/ARE pathway. Environ Sci Pollut Res 26:34575–34583

Yang S-H, Yu L-H, Li L, Guo Y, Zhang Y, Long M, Li P, He J-B (2018) Protective mechanism of sulforaphane on cadmium-induced Sertoli cell injury in mice testis via Nrf2/ARE signaling pathway. Molecules 23:1774

Zang H, Mathew RO, Cui T (2020) The dark side of Nrf2 in the heart. Front Physiol 11:722

Zhang Y, Cao Y, Wang F, Song M, Rui X, Li Y, Li C (2016) 4-Nitrophenol induces activation of Nrf2 antioxidant pathway and apoptosis of the germ cells in rat testes. Environ Sci Pollut Res 23:13035–13046

Zhang Y, Piao Y, Li Y, Song M, Tang P, Li C (2013) 4-Nitrophenol induces Leydig cells hyperplasia, which may contribute to the differential modulation of the androgen receptor and estrogen receptor-α and-β expression in male rat testes. Toxicol Lett 223:228–235

Zhao Y, Li M-Z, Shen Y, Lin J, Wang H-R, Talukder M, Li J-L (2019) Lycopene prevents DEHP-induced Leydig cell damage with the Nrf2 antioxidant signaling pathway in mice. J Agric Food Chem 68:2031–2040

Zhao Y, Li M-Z, Talukder M, Luo Y, Shen Y, Wang H-R, Li J-L (2020) Effect of mitochondrial quality control on the lycopene antagonizing DEHP-induced mitophagy in spermatogenic cells. Food Funct 11:5815–5826

Zong S, Pu Y, Li S, Xu B, Zhang Y, Zhang T, Wang B (2018) Beneficial anti-inflammatory effect of paeonol self-microemulsion-loaded colon-specific capsules on experimental ulcerative colitis rats. Artif Cells Nanomed Biotechnol 46:324–335