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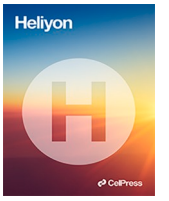

Review article

Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling in heavy metals-induced oxidative stress

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ABSTRACT

Organisms encounter reactive oxidants through intrinsic metabolism and environmental exposure to toxicants. Reactive oxygen and nitrogen species (ROS, RNS) are generally considered detrimental because they induce oxidative stress. In order to combat oxidative stress, a potential modulator of cellular defense nuclear factor erythroid 2-related factor 2 (Nrf2) and its endogenous inhibitor Kelch-like ECH-associated protein 1 (Keap1) operate as a common, genetically preserved intrinsic defense system. There has been a significant increase in the amount of harmful metalloids and metals that individuals are exposed to through their food, water, and air, primarily due to human activities. Many studies have looked at the connection between the emergence of different ailments in humans and ecological exposure to metalloids, i.e., arsenic (As) and metals viz., chromium (Cr), mercury (Hg), cadmium (Cd), cobalt (Co), and lead (Pb). It is known that they can produce ROS in several organs by both direct and indirect means. Studies suggest that Nrf2 signaling is a crucial mechanism in maintaining antioxidant balance and can have two roles, depending on the particular biological setting. From one perspective, Nrf2 is an essential defense mechanism against metal-induced toxicity. Still, it may also operate as a catalyst for metal-induced carcinogenesis in situations involving protracted exposure and persistent activation. Therefore, this review aims to provide an overview of the antioxidant defense mechanism of Nrf2-Keap1 signaling and the interrelation between Nrf2 signaling and the toxic elements.

1. Introduction

It has been determined that a key aspect of organismal health is the connection between the environment and the biological framework. Exposure to heavy metals, pesticides, dyes, and plastics can lead to biological and ecological imbalances and severe health risks for humans. The elements known as metals and metalloids, or heavy metals overall, have densities five times greater than those of universal solvent i.e. water. Natural phenomena, including soil erosion, rock weathering, forest fires, and volcanic eruptions, release heavy metals [1] in the surrounding environment. Heavy metals naturally find their way into water bodies through stream runoff in addition to being present in the earth's core. While heavy metals are naturally absorbed by the environment, it is crucial to note that a range of human activities significantly increase the ambient concentration of heavy metals, thereby contributing to the environmental and health risks. Ecological compartments get contaminated due to this unwarranted rise in the level of heavy metals by the waste

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dump, discharge, smoke, and industries such as textiles, metal processing, smelting, plastics, mining, medicines, and wood and paper processing. The primary sources of soil and groundwater contamination are agricultural practices such as using fertilizers, pesticides, and manures [2]. Additionally, heavy metals are released into the air, soil, and water from home sources like fuel burning and sewage.

In addition to existing in many environmental sections, trace amounts of heavy metals are also found in live organisms and contribute to various biological activities. For instance, zinc (Zn) is not just structurally important but also plays a regulatory role, copper (Cu) is almost present in all tissues and is necessary for several metabolic processes, while iron (Fe) is absolutely critical for oxygen transport and nucleic acid synthesis [3]. The roles of selenium (Se), cobalt (Co), and chromium (Cr) are in glucose metabolism, vitamin B12 production, and antioxidant defense mechanisms, respectively [3].

A growing body of research indicates that heavy metals in the biological system interact with numerous well-characterized and poorly-defined cellular components and processes to produce toxicity. Exposure to heavy metals results in cellular toxicity, which can have an organismal impact through developmental deformities, behavioral changes, abnormal reproductive outcomes, and shortened life spans [4,5]. However, the impact of toxicants in various organisms may differ depending upon the pathway and sequence of exposure, buildup, and metabolism, as well as target and non-target organ toxicity [6,7]. The body constantly generates reactive oxygen and nitrogen species (ROS, RNS) as a result of both internal metabolic processes and external stimuli [8]. Reactive oxidants play a significant role in controlling physiological and pathological consequences in organisms ranging from prokaryotes to humans. Reactive oxidants are created in a regulated manner, with some even having beneficial functions in normal cells. Oxidants produced in reaction to physiological stimuli have a crucial role as signaling molecules in regulating various physiological processes, including autophagy, inflammation, immunological response, and differentiation of cells [9]. According to recent research, one of the crucial factors inducing the pathophysiology of several health problems, including cancer, neurodegeneration, inflammation, developmental disorders, and reproductive diseases, is elevated oxidative stress [10–12]. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) are examples of enzymatic and non-enzymatic antioxidants that are important for preserving redox equilibrium through regulating ROS production. The elements of the first line of defense against oxidative stress include SOD, CAT, and GPx. SOD is a metalloenzyme that has an occupied active center by Cu and Zn and occasionally by Fe or manganese (Mn). Superoxide radicals are converted into oxygen (O_2) and hydrogen peroxide (H_2O_2) by SOD. H_2O_2 is broken down into O_2 and water (H_2O) by CAT and GPx.

ROS plays a critical function in controlling the signaling pathways connected to different stress situations. Depending on the type of stress, ROS may operate as a downstream effector or an upstream activator of various cellular signaling [13]. The multifaceted eukaryotic system, however, makes the interaction between the ROS and signaling route complex and occasionally contentious. In toxicology, the xenobiotic reaction is determined by the interplay between ROS and cellular signaling. ROS can interact at the transcriptional or translational level with pathways that lead to either cell death or survival, depending on the situation. Over the past ten years, researchers have focused on understanding how signal transduction and ROS interact to cause cellular and organismal responses to heavy metals. The nuclear factor erythroid 2–related factor 2 (Nrf2) is a master regulator of the cellular defense machinery to keep oxidant-antioxidants levels in control for maintaining cellular equilibrium. Nrf2 regulates the basal and induced expression of antioxidant genes to modulate the oxidant levels for the cellular homeostasis. The purpose of this study is to provide an overview of current understanding of the Nrf2 signaling by which it exerts its protective effect against these heavy metals and metalloids.

1.1. Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling

The gene nuclear factor erythroid 2-like 2 (NFE2L2) encodes Nrf2, identified by Moi et al. [14], is one of the cap “n" collar basic region-leucine zipper transcription regulators. It was subsequently reported to be a primary sensor of oxidative stress in cells [15,16]. The cytoplasmic location of Nrf2 under normal circumstances is linked with Kelch-like ECH-associated protein 1 (Keap1).

Seven functional domains (Neh1–7) of Nrf2 were identified through domain analysis using nuclear magnetic resonance spectroscopy and high-resolution crystal structure. These domains are important in the control of either the transcriptional process (transactivation) or stability. Whereas the Neh5 domain controls Nrf2’s intracellular localization, the N-terminal domain is in charge of the interaction between Nrf2 and Keap1 at lower nanomolar concentrations (KD∼5 nM), stability of Nrf2, and ubiquitination [17,18]. The β-transducin repeat-containing protein binds to the Neh6 domain, which also regulates Keap1-independent Nrf2 degradation. Because of its fundamental leucine zipper motif, the Neh1 domain facilitates Nrf2’s binding to the antioxidant response element (ARE) sequence. Additionally, this domain can regulate the equilibrium of the Nrf2 protein by associating with ubiquitin-conjugating enzyme E2 (UbcM2) [19]. The nuclear positioning signal that the Neh1 region shows after being released from Keap1 is required for the nuclear movement of Nrf2. The transcription co-activator chromo-ATPase/helicase DNA-binding protein (CHD6) engages with the C-terminal of the Neh3 domain to cause the transactivation of ARE-dependent genes following chromatin remodelling [17,18,20]. The co-activator cyclic adenosine monophosphate-responsive element-binding protein is bound by the transcription activation domains Neh4 and Neh5, which in turn promotes Nrf2 transcription [20]. Neh4 and Neh5 have the ability to improve Nrf2-targeted ARE gene expression through interactions with the different nuclear cofactors [17,20]. Repressing Nrf2, the Neh7 domain binds with retinoic X receptor α [21]. Keap1, the primary intracellular controller of Nrf2, is distinguished by five domains, namely two glycine repeat domains (DGR), one intervening region (IVR), and three broad complex-tramtrack-bric a brac (BTB). Each domain plays a crucial role in reducing Nrf2 activity. The Keap1 homodimer’s DGR domains link to the ETGE (hinge) and DLG (latch) regions with varying affinities in a single Nrf2 molecule (the concept of hinge and latch) [19].

The Cul 3–Rbx1–E3 ubiquitin ligase combination constantly stimulates Nrf2 for proteasomal breakdown by connecting an individual Nrf2 protein and a Keap1 homodimer [22]. Two distinct processes release Nrf2 from Keap1 downstream regulation after stimulation-induced phosphorylation [23,24]. In the first process, known as the “canonical pathway,” highly reactive cysteines found

in Keap1 domains are chemically modified. These modifications result in protein-protein crosslinks formed after reacting with electrophiles, which disrupt Nrf2's connection with the Cul3–Keap1 E3 ubiquitin ligase complex and reduce Nrf2 protease complex breakdown [25,26]. The second process, referred to as the "non-canonical pathway," involves several proteins, including the X chromosomes Wilms tumor gene (WTX), dipeptidyl peptidase III (DPP3), and others. By interacting with Keap1 or Nrf2 for reciprocal binding, the proteins can obstruct the development of the Nrf2-Keap1 unit, which in turn reduces Nrf2 ubiquitination while enhancing its nuclear translocation and stimulation [26].

Remarkably, several more mechanisms that regulate Nrf2 equilibrium apart from Keap1 have been reported to date. First, glycogen synthase kinase-3 (GSK-3) phosphorylates Nrf2 in its serine-rich Neh6 domain, making it easier for β-transducin repeat-containing protein (β- TrCP) to recognize Nrf2. TrCP is a substrate interface for the Skp1–Cul1 ubiquitin ligase complex, which activates Nrf2 for ubiquitination and protease complex breakdown [27]. However, another mechanism works by means of the autophagy–lysosome pathway, which is typically engaged in eliminating harmed subcellular constituents, such as proteins and organelles. The sequestosome 1 (p62/SQSTM1), a multi-region molecule that associates with several molecules inside a single Nrf2 molecule, accumulates in conditions that promote autophagic failure [28,29]. Among other functions, p62 binds with Keap1 and sequesters it inside autophagosomes to stop Nrf2 from recognizing it [28]. The E3 ubiquitin ligase 3-Hydroxy-3-methylglutaryl reductase degradation (HRD1) often used to control protein throughput in the ER-associated degradation (ERAD) pathway, is a key component of another significant mechanism at the junction of ER and oxidative stress-stress response [30]. Nevertheless, it has been demonstrated that HRD1 is overexpressed during ER stress in cirrhotic livers and adversely regulates NRF2 stability by encouraging its ubiquitination and eventual destruction [30]. These mechanisms are believed to accurately regulate the degree of Nrf2 cascade stimulation, preventing the unnecessary upregulation of downstream target genes that result from Nrf2 eluding protease complex breakdown and entering the nucleus. Nrf2 attaches itself to the Maf proteins after translocation into the nucleus [23]. Many detoxifying and antioxidant genes are favorably regulated by Nrf2-Maf heterodimers. These genes, which include NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), and glucose-6-phosphate dehydrogenase 1 (G6PD), enable NADPH re-generation and provide immunity to oxidative stress and xenobiotics, [31]. Other target genes viz., CAT, glutathione reductase (GSR), GSH-Px, thioredoxin (TRX), glutaredoxin (GRX), involved in antioxidant activity are further initiated transcriptionally by the Nrf2/ARE pathway [32].

The Nrf2/Keap1 signaling system regulates oxidative stress events [33] which are important in causing inflammation [34], pre-eclamptic pregnancies [35], and cancer [36]. In a recent review, authors have shown the importance of Nrf2 in maintaining cellular homeostasis in the biological system. Authors have emphasized on a dual role of Nrf2 depending on the biological relevance viz., as an anti-oxidant in metal-induced toxicity, while triggering carcinogenesis upon continuous activation due to metal induced-oxidative stress and have summarized the functionality between Nrf2 signaling and metals associated toxicity on exposure [37]. It is worth noting that elevated levels of ROS can result in cellular demise, whereas lower levels have been linked to the development and advancement of cancer. Contrarily, elevated levels of Nrf2 can counteract the effects of ROS, helping to maintain redox homeostasis and promote cell survival [38]. In addition, levels of ROS are more commonly elevated in cancer cells compared to normal cells [39]. It is possible that this is a result of the increased metabolism of cancer cells in comparison to normal cells [40]. As a result, the Nrf2/Keap1 pathway is a newly discovered target for chemotherapy and radiation therapy in a number of cancer types, including prostate cancer [41], cervical and endometrial cancer [42]. According to reports, Nrf2 expression was actually much higher in cancer tissues that were resistant to chemotherapy and radiation therapy, shielding the cells from the oxidative damage brought on by these treatments [42,43]. Furthermore, it has been documented that Nrf2 can activate the expression of ATP-binding cassette (ABC)

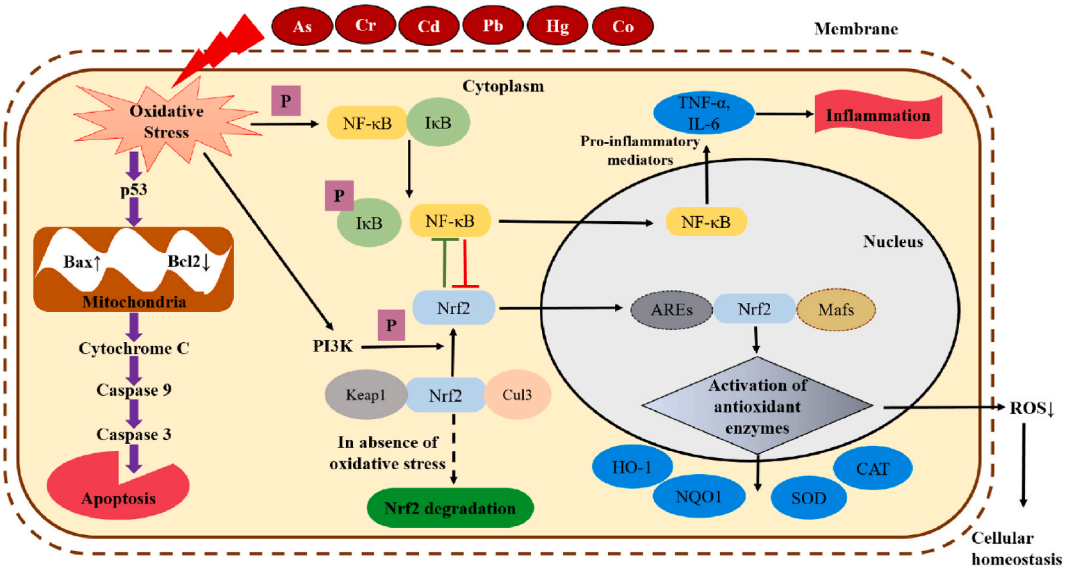

Fig. 1. A schematic diagram of the mechanism of action of Nrf2 signaling in response to oxidative stress.

transporters, which further shields cancer cells from the drug-expelling effects of chemotherapy [44]. Moreover, Nrf2 promotes the tumorigenicity and chemo resistance of cancer stem cells (CSCs) by playing a crucial role in their survival and ability to self-renew [43]. Author has also reviewed that both natural and artificial compounds that can lower Nrf2 [45] and NQO-1 [46] expression in cancer cells have the potential to greatly enhance therapeutic efficacy and improve patient outcomes. It also serves as a xenobiotic-activated receptor (XAR) that controls the adaptive reaction, providing a potent defense barrier for living beings against being subjected to ecological toxins [47].

It is known that a wide range of hazardous chemicals, including paraquat [48], phthalates [49], aflatoxin B1 [50], and toxic metals and metalloids including Cr [51], Pb [52], and As [53] can disrupt Nrf2. Furthermore, it has been demonstrated that As, Pb, and Cr separate the Nrf2-Keap1 complex. These harmful chemicals can identify and alter certain cysteine residues, including Cys 226 and Cys 613, on the surface of Keap1. Following this, the Keap1 molecule develops several sensor systems and experiences a disruption in its function, which in turn causes Nrf2 depression/activation. However, since it has been demonstrated that the Nrf2 pathway protects against external oxidative damage, numerous recent researches have looked for a protective chemical that may cause particular Nrf2 activation. Consequently, it has been demonstrated that several phytochemicals, including biochanin-A (BCA) [54], coenzyme Q10 (CoQ10) [55], phloretin (PHL) [56], epigallocatechin gallate (EGCG) and quercetin [57], to cite a few, reduce metal-associated toxicity via altering the Nrf2-dependent cell defense systems [58–61]. Fig. 1 A schematic diagram of the mechanism of action of Nrf2 signaling in response to oxidative stress.

1.2. Heavy metals induced alterations in Nrf2 signaling

Arsenic (As) - Both natural and man-made activities release arsenic into the ecosystem. It can be found in trivalent (As [III]) and pentavalent (As [V]) states, and compared to the organic state, the inorganic state is considered more hazardous and carcinogenic. Chronic arsenic exposure has been related to lung fibrosis, chronic obstructive pulmonary disease (COPD), and other respiratory illnesses. It has also been proven to have various harmful consequences and disturb normal cellular equilibrium [62].

The Nrf2 signaling pathway is crucial in preventing and treating arsenic toxicity and plays a significant role in the antioxidation process. However, chronic exposure to arsenic can lead to increased activity of the Nrf2 pathway, which promotes cell proliferation and carcinogenicity [63]. Aono et al., was the first group who suggested that Nrf2 signaling has a role in arsenic toxicity, and osteoblasts exposed to 800 μM of As (V) for 16 h activated Nrf2 and target proteins such as HO-1, Peroxiredoxin I (Prx I) and stress protein (A170) as one of the protective mechanisms required for cell viability [64]. Additionally, the authors in another study demonstrated that following arsenic exposure, Nrf2 is necessary for initiating the detoxifying gene, NQO1, in mouse hepatoma cells [65]. Consequently, an *in vivo* study has suggested that Nrf2 may have a protective function against oxidative stress in *Swiss* albino mice. Exposure to arsenic led to increased oxidative stress, while treatment with various antioxidants upregulated Nrf2 gene expression, restoring cellular homeostasis [54]. Studies showed that mice exposed to arsenic elevated the alanine aminotransferase (ALT) aspartate aminotransferase (AST), and malonaldehyde (MDA) levels, together with the decline in GSH levels. Following three months of arsenic treatment, mice lungs also showed a rise in the synthesis of SOD2 and HO-1, which was connected to the increased Nrf2 protein expression [66]. Rats exposed to 5 mg/kg arsenic trioxide (As_2O_3) showed increased MDA and ROS levels and decreased activity of Nrf2, NQO1, and HO-1 along with alterations in interleukin 6 and 1β (IL-6, IL-1β), and tumor necrosis factor-alpha (TNF-α) [67].

Li et al., demonstrated that exposure to arsenic increased the levels of MDA, ROS accumulations, and the GSSG/GSH ratio in both *in vivo* and *in vitro* experimental models. Conversely, the decreased activity of SOD, CAT, and GSH-Px, in addition to total sulfhydryl groups (TSH) and GSH, were shown. Subsequent investigation revealed that arsenic dramatically increased the apoptosis by down-regulating B-cell lymphoma (Bcl-2) expression together with upregulation of Bcl-2-associated X protein (Bax), Cyt C, p53, caspase 3 (Cas 3), and caspase 9 (Cas 9) expressions along with the decreased expression of Nrf2, HO-1, and NQO1 [68]. Another study revealed that As_2O_3 exposure drastically lowered the longevity, migration, and invasion of 5-FU-resistant Hep3B cells when combined with Nrf2 knockdown. It also deactivated the signaling of hypoxia-inducible factor-1α (HIF-1α) and heat shock protein 70 (HSP70), impeded Bcl-2 activity, and elevated the expression of Bax and Cas 3 [69]. As_2O_3-treated H9c2 cardiomyocytes decreased the mitochondrial transmembrane potential (MTP) and total antioxidant capacity (TAC), according to an *in vitro* investigation. Also, it was found that when As_2O_3 was administered, the activity of the transcription factors Nrf2 and Bcl2 changed, increasing the aggregation of calcium in cells and the production of ROS [70]. In a study by Wang et al., it was observed that human uroepithelial cells (SV-HUC-1) cells exposed to sodium arsenite ($NaAsO_2$) (1–10 μM) elevated the level of ROS generation, GSH, cyclooxygenase-2 (COX-2), and Nrf2 [71]. Another study in the recent past showed that treatment with As_2O_3 (10 μM) indicated a rise in LPO and reduction in antioxidant status, MTP, and TAC, along with altered Nrf2 and Bcl2 expressions [72].

Chromium (Cr) – Trivalent (Cr [III]) and hexavalent (Cr [VI]) forms of the transition metal Cr can be found in nature. Due to its greater mobility and solubility than in its trivalent state, hexavalent state has more widespread contamination of soil, water, and air, further impairing human health [73]. In low quantities, the Cr [III] is regarded as an indispensable micronutrient that is necessary for the metabolism of proteins, fats, and carbohydrates. Conversely, it has been discovered that Cr [VI] may be a toxin and carcinogen that increases the chance of cancer, neurological impairments, skin allergies, and reproductive problems [74]. Cr [VI] has a very high permeability and may readily penetrate the plasma membrane through sulphur anion transport system to form intermediate species, Cr [V], Cr [IV], which is metastable, and Cr [III], which is the most stable [75].

According to a study [76], Cr [VI] at a concentration of 2 mg/L for 60 days caused neurotoxicity in zebrafish as Cr passes across the blood brain barrier (BBB) resulting in the increase in CAT activity and MDA content. It was evident from the upregulation of Nrf2, NQO1, and HO1 that the Nrf2-ARE system was involved in the stress response against the neurotoxicity produced by Cr [VI]. In another study, rats exposed to a single dosage of potassium dichromate ($K_2Cr_2O_7$), showed that Nrf2 signaling had a significant role in

regulating ROS-induced cytotoxicity by protecting the hepatocytes and activating antioxidant enzymes [77]. Moreover, Shaw et al., showed that even environmentally significant Cr concentrations may start an upsurge in Nrf2 at the transcriptional and translational levels in zebrafish, as well as boost its nuclear translocation [78]. Numerous investigations have shown that exposure to Cr, either alone or in conjunction with arsenic, is associated with increased formation of ROS and cholinesterase (ChE) activity. The findings of the studies have established Nrf2 pathway as a critical mechanism for neural protection against oxidative stress [56]. In a recent study by Wang et al. [79], it was found that broilers subjected to Cr [VI] altered the liver-somatic index (LSI), biochemical indices, Nrf2 pathway linked factors, and autophagy-associated genes.

The impact of Co-Cr dental alloys on human gingival fibroblasts (HGF) and osteoblasts were examined using an *in vitro* model. The authors found that, Co-Cr alloys activate Nrf2/ARE signaling and upregulate HO-1 to cause cytotoxicity and inflammatory responses [80]. In another study, Cr [VI] intoxication in a dose dependent manner for 24 h exhibited cytotoxic effects in cultured human liver hepatoblastoma (HepG2) cells. Exposure to Cr [VI] not only caused oxidative damage but also stimulated Cas 3. Furthermore, Cr [VI] increased the expression of Nrf2-dependent antioxidant enzymes, such as SOD2, glutamate-cysteine ligase catalytic subunit (GCLC), and HO1, along with translocation of Nrf2 into the nucleus [81].

Mercury (Hg) - It is a common ambient hazardous element that, at low concentrations, can have serious harmful effects on a number of organs. Mercury is a highly bioaccumulative and poisonous metal. Because of its detrimental effects on the marine ecosystem, a lot of research is done on the distribution of mercury in the marine ecosystem [82]. Human activities like mining, incineration, agricultural sector, release of municipal and industrial effluents are the major causes of mercury pollution. It easily crosses the BBB and disrupts the central nervous system, particularly its organic form viz., methylmercury (MeHg).

Research conducted *in vivo* has validated the connection among oxidative stress, Nrf2, and mercury exposure in different organs. A study showed that mice injected with single dose of mercuric chloride ($HgCl_2$) upregulated the ALT and AST levels along with the inflammatory changes. It was also found that Hg increased the oxidative stress in mice which can be linked with cytochrome P450 (CYP450) and also downregulated the Nrf2/HO-1 signaling in the liver of mice. Another study with similar dose of $HgCl_2$ in mouse triggered lung injury with increased oxidative stress. Hg administration altered the production of cytokines, activation of neutrophils, histological changes, and increased apoptosis. It was found that Hg decreased the protein kinase B (AKT) phosphorylation and Nrf2 signaling and activated the nuclear factor kappa B (NF-κB) in lung tissue of mouse [83]. Similarly, $HgCl_2$ (80 mg/L, in drinking water) induced cardiac histological changes along with oxidative stress and apoptosis. Also, phosphatidylinositol 3-kinase (PI3K), AKT, Nrf2, and its downstream proteins were found to decrease along with activation of NF-κB in the heart of rats treated by $HgCl_2$ [25].

The cells react to organic mercury, specifically MeHg, by activating Nrf2 in conjunction with S-mercuration of Keap1. It's crucial to remember that Keap1 is a cysteine-rich protein with 25 cysteine residues and therefore, it is plausible that MeHg alters Keap1 by S-mercuration of certain cysteines and triggers Nrf2, HO-1, and GCLC. The study showed that MeHg binding to Keap1 activated Nrf2 in human neuroblastoma (SH-SY5Y) cells after exposure to MeHg in primary mouse hepatocytes isolated from Nrf2-deficient mice. Also, MeHg-induced cytotoxicity was reduced by Nrf2 overexpression [82]. Another study revealed that the synthesised ethyl monoester of the MeHg-SG adduct caused concentration-dependent cell injury in SH-SY5Y cells, which triggered Nrf2 and consequently enhanced the downstream genes [84]. In a different investigation, it was discovered that MeHg increased the mRNA expression of NQO1 in hepa-1c1c7 cells exposed to varying MeHg concentrations in a way that was both time- and dose-dependent. Moreover, the rise in Nrf2 protein's nucleus localization coincided with the MeHg-mediated upregulation of NQO1 expression.

Cadmium (Cd) – Cd is a common industrial and environmental contaminant. The two main ways the general population is exposed to Cd are through tobacco usage and food. After human consumption, it continues to build up inside their bodies for the duration of their lives. This metal was first employed as a paint industry pigment and as a tin substitute during World War I [85]. These days, it is also utilized in the creation of specific alloys and rechargeable batteries. Because Cd transfers from the soil to plants at a high pace, it is primarily found in fruits and vegetables. Cd is widely acknowledged for its detrimental effects on intracellular enzymatic structures, oxidative stress and inadequate nutrition in plants [86].

The majority of the research findings are consistent with the theory that exposure to Cd regulates Nrf2, inhibits its disintegration, and stimulates its nuclear relocation as a form of defense. Adult zebrafish exposed to 24 h of Cd, showed a dose-dependent stimulation of Nrf2-regulated genes linked to intracellular reactions to oxidative damage in their olfactory system. Increased mRNA levels of GST, GCLC, HO-1, and Prx 1 in zebrafish larvae exposed to Cd for 3 h were suggestive of Nrf2 activation [87]. By altering thyroid follicular morphology and endocrine function, exposure to Cd for 4 and 8 weeks may lower body weight and cause thyrotoxicity. This is also associated with increased oxidative stress and apoptosis, macrophage infiltration, and inflammatory cytokine release. Cd increased the production of nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3), Caspase-1, IL-1β, and IL-18, and could hinder the antioxidant system by upregulating Keap1, the antioxidant pathway's negative feedback regulator, and downregulating Nrf2, the antioxidant response protein in mice [88]. Cd exposure for 7 days decreased the follicle stimulating hormone (FSH), testosterone, and luteinizing hormone (LH) levels in mice. Alternatively, increase in LPO, nitrate/nitrite levels, and GSH depletion indicated a disruption in the redox balance in the testicular tissue. Additionally, there was an inhibition of Nrf2, CAT, glutathione reductase (GR), SOD, and GPx activity and gene expression. TNF-α and IL-1β levels were also raised and Cas 3 and Bax were upregulated together with downregulating Bcl-2, setting off an apoptotic cascade [89].

Lawal and Ellis (2011) hypothesized that Cd stimulates phospholipase C by engaging with a particular G protein-coupled metal-binding receptor in macrophages and causing nuclear Nrf2 activation. Intracellular Ca^{2+} and diacylglycerol are released as a result of this occurrence, and both of these substances have the ability to alter Nrf2 signaling in astrocytoma cell [90]. Through the use of Nrf2 knockout (Nrf2−/−) mouse embryonic fibroblast cells, it was demonstrated that Nrf2's elimination is linked with a major rise in ROS generation, thus, increasing the susceptibility to Cd-induced cell death. The Keap1–Nrf2 system and the overexpression of metal-lothioneins (MTs) may bind Cd, and it's been shown by *in vitro* tests on bovine aortic endothelial cells. This provides additional

evidence of the beneficial effects of Nrf2 signaling against Cd toxicity, as MTs activation acts as initial reaction by ensnaring and immobilizing Cd via the cysteine residues on the MTs [91]. Wu et al., demonstrated that Nrf2 stimulation prevents Cd-induced oxidative stress and liver damage through upregulation of genes involved in antioxidant defense system in the liver of mice following a single dosage of cadmium chloride ($CdCl_2$) [92].

Table 1
Summary of the effects of various metals/metalloids *in vitro* models on Nrf2 signaling and its interrelation with oxidative stress parameters.

Metals/ Metalloids	Experimental model	Dose	Duration	Results	Reference
Arsenic	LO2 cell line	(0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20) ppm	6, 12, 24, 48, 72 h	↓ (SOD, CAT and GSH-Px, TSH, GSH, Bcl-2, Nrf2, HO-1, NQO1), ↑ (ROS, MDA, Bax, p53, Cyt C, Cleaved Cas-3 and 9)	[68]
Arsenic	SNU-387, SNU-449, Huh 7 and Hep3B cells	(0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128) μM	72 h	↑ (Nrf2 in cell lines), ↓ (HIF-1α/HSP70, Bcl2) and ↑ (Bax, Cas 3) when coupled with Nrf2 knockdown	[69]
Arsenic	H9C2 cardiomyocytes	10 μM	48 h	↓ (TAC, MTP, Nrf2, Bcl2), ↑ (ROS)	[70]
Arsenic	SV-HUC-1 cells	0–20 μM	24 h	↑ (COX2, GSH, ROS, Nrf2, ERK, p38)	[71]
Arsenic	Chang human hepatocytes	10 μM	48 h	↑ (LDH, LPO, ROS), ↓ (TAC, GSH, CAT, SOD, MTP, Nrf2)	[72]
Arsenic	MC3T3-E1 osteoblasts	As(V) -(200–800 μM) As(III) -(50–100 μM)	16 h	As(V) induced significant ↑ in Nrf2 within 4 h, maximizing at 16 h and remaining raised up to 24 h, ↑HO-1, ↑Prx 1. As(III) delayed ↑ in Nrf2 levels, reaching a maximum at 12 h and consequently declining to control levels after 24 h	[64]
Arsenic	Human keratinocyte cell line (HaCaT)	100 nM	28 weeks	↑ (MMP-9, keratin-1, keratin-10, GSH, Nrf2, HO-1, NQO1)	[53]
Arsenic	Non-small cell lung cancer (NSCLC) cell lines	0.5 μM	3 months	↑ invasive and migration of cells, ↑ Nrf2 dependent SOX-9, ↑Nrf2 gene via knockout of Keap1	[63]
Chromium	HGF cell line and human osteoblasts	Wt% (Co- 65.2 Cr- 27.2) alloy	24 or 72 h	↑ (ROS, TNF-α, IL-1β, IL-6, IL-8, COX2, iNOS, JAK2/STAT3, p38/ERK/JNK MAPKs, NF-κB, Nrf2, HO-1)	[80]
Chromium	Human liver (HepG2) cells	(0, 3.125, 6.25, 12.5, 25, 50) μM	24 h	↑ (ROS, MDA, Nrf2, SOD2, GCLC, HO1)	[81]
Chromium	H9C2 cell (rat myocardial cell)	5.5 μg/mL	24 h	↓ (Nrf2, AMPK, cell viability)	[51]
Mercury	SH-SY5Y cells	Adduct of MeHg (125 mg or 20 mg) ethyl monoester of GSH (13.7 mg)	30 min	↑ (Nrf2 and its downstream genes) and modified protein thiols together with Keap1	[84]
Mercury	Hepa-1c1c7 cells	5 μM	0, 6, 12, 24 h	↑ (Nrf2, NQO1)	[106]
Cadmium	Astrocytoma cell line 1321N1	(5 and 10) μM	24 h	↑ (HO-1, NQO1, nuclear aggregation of Nrf2) and Association of PKC in the Nrf2-mediated response of cells	[90]
Cadmium	Nrf2 knockout (Nrf2−/−) mouse embryonic fibroblasts (MEF) cells	(2, 5, 10, 50, 100) μM	5 h	↑ (ROS, sensitivity to Cd-induced cell death in Nrf2 knockout cells.	[107]
Cadmium	Bovine aortic endothelial cells	(0.5, 1, 2, 5) μM	24 h	↑ (MTs), Alteration of cysteine in Keap1 and Nrf2 stimulation	[91]
Cobalt	PC12 cells	0.1–1 mM	24 h	↑ (ROS, Bax, cleaved cas 3 and 9, PARP), ↓ (GSH, Bcl2, Nrf2)	[96]
Lead	PC12 cell line	(5, 10, 20, 30) mM	24 h	↑ (Bax/Bcl2, Cleaved Cas3), ↓ (Nrf2, CAT, MnSOD)	[99]
Lead	Bovine granulosa cells	(1, 2, 3, 5, 10) μg/mL	2 h	↑ (ROS, PCC, GRP78, CHOP C/EBP homologous protein, Cas 3), ↓ (Nrf2, NF-kβ, Bcl2)	[100]
Lead	Rat primary microglia and astrocytes	(0, 5, 15, 30, 50 and 100) μmol/L	24 h	↑ (ROS, Nrf2), ↓ (GSH) in microglia than astrocytes	[103]
Lead	SH-SY5Y cells	125 μM	(3, 6, 12, 24) hrs	↑ (Nrf2, Nuclear Nrf2 aggregation, HO-1, GSTα1, GCLM, GCLC, NQO1)	[104]
Lead	SH-SY5Y human neuroblastoma cells	(0, 5, 25, 125) μmol/L	12 h	↑ (Bax, Cyt C, Cas3, Bcl-2), ↓ (Nrf2)	[105]

MMP-9- Matrix metallopeptidase 9.
SOX 9- SRY-Box Transcription Factor 9.
iNOS- Inducible nitric oxide synthase.
JAK/STAT- Janus kinase/signal transducers and activators of transcription.
PKC- protein kinase C.
PARP- Poly (ADP-ribose) polymerases.
GRP78- Glucose-regulated protein 78.
CHOP- C/EBP homologous protein.

Cobalt (Co) - The two valence states of cobalt that are most common in cobalt compounds are cobaltous (Co^{2+}) and cobaltic (Co^{3+}), with the former being the more ecologically and commercially accessible [93]. Cobalt's only biological significance is as a metal in vitamin B12, commonly known as cyanocobalamin. Other cobalt compounds, on the other hand, have been reported to be hazardous to humans and the environment when exposed in excess [94]. Due to its extensive presence, different Co compounds are regularly encountered by humans in their daily lives. The main ways that the general public is exposed are by breathing in ambient air, consuming food, and ingesting water that contains Co compounds. The greatest systemic Co concentrations are obtained from internal exposure via metal-on-metal (MoM) hip implants and oral Co supplementation [94].

Table 2
Summary of the effects of various metals/metalloids *in vivo* models on Nrf2 signaling and its interrelation with oxidative stress parameters.

Metals/ Metalloids	Experimental model	Dose	Duration	Results	Reference
Arsenic	*Swiss* albino mice	20 mg/kg bw/day	2-week	↓ sperm motility, ↑ accumulation in kidney and liver, ↓ (SOD, GSH, GST), ↑ (LPO, PCC, CAT, Nrf2)	[54]
Arsenic	Kunming mice	(10,50, 100) mg/L	6 weeks	↑ (ALT, AST, MDA), ↓ (GSH, Nrf2, HO-1, NQO1)	[108]
Arsenic	C57BL/6 mice	10 mg/kg bw/day	14 days	↑ (ALT, AST, MDA), ↓ (Nrf2, NQO1)	[109]
Arsenic	*Swiss* albino mice	10 mg/kg	3 months	↑ accumulation in lungs, ↑ (ROS, LPO, PCC, Bax/Bcl2), ↓ (SOD, CAT, GST, GR and GPx, Nrf2, HO-1, SOD2)	[66]
Arsenic	*Sprague* Dawley rats	5 mg/kg	7 days	↑ (ALT, AST, ALP, MDA, ROS), ↓ (CAT, SOD, Nrf2, HO-1, NQO1)	[67]
Arsenic	ICR mice	5 mg/kg	7 days	↑ (ALT, AST, MDA, ROS, Cleaved-Cas 3, 9, p53, CytC, Bax/Bcl-2, Cas 3, 9), ↓ (SOD, CAT, GSH-Px, TSH, GSH, Nrf2, HO-1, NQO1)	[68]
Chromium	Zebrafish (*Danio rerio*)	2 mg/L	1, 7, 15, 30, 60 days	↑ (GSH, MDA, CAT, Nrf2, HO-1, NQO1, Bax, Cas 9, Cas 3, AChE), ↓ (Bcl2)	[76]
Chromium	*Wistar* rats	17 mg/kg bw	12 and 24 h for 3 days	↑ (ALT, AST, LDH, LPO, ROS), ↓ (SOD, CAT, GPx, Nrf2)	[77]
Chromium	Wild-type zebrafish	2 mg/L	1, 7, 15, 30, 60 days	↑ (ROS, GSH, MDA, CAT, Nrf2, Cyp1a, Cu/Zn SOD, ↓ (HO-1, Hsp 70, Ucp2)	[78]
Chromium	*Swiss* albino mice	75 ppm	30 days	↑ (PCC, MDA), ↓ (GSH, SOD, GST, TT, CAT, Nrf2, HO-1, NQO1)	[56]
Chromium	*Swiss* albino mice	Cr-75 ppm + As-100 ppm in combination	15 days	↑ (PCC, MDA), ↓ (GSH, SOD, GST, TT, Nrf2, AChE, BChE)	[55]
Chromium	Broilers	37 mg/kg bw	42 days	↑ (ALT, AST, MDA, Beclin 1, p62), ↓ (GSH, SOD, GPx-1, Nrf2, HO-1, NQO1, mTOR)	[79]
Chromium	*Wistar* rats	4 mg/kg	35 days	↓ (AMPK, Nrf2, HO-1, NQO1, Bcl2), ↑ (NF-kβ, Cleaved Cas 3, p53)	[51]
Mercury	Kunming mice	5 mg/kg	At 24th hr once	↑ (MPO, MDA, TNFα, IL-1β, IL-6, Bax, Cas3), ↓ (GSH, SOD, Bcl2, Nrf2, HO-1)	[83]
Mercury	*Wistar* rats	80 mg/L	56 days	↑ (Hg accumulation in blood and heart, MDA, TNFα, Cleaved Cas 3, p53, Bax), ↓ (GSH,Nrf2, HO-1,NQO1, Bcl2, P-AKT)	[25]
Cadmium	Zebrafish	Adult (0, 11, and 110) μg/L Embryo (0, 562 and 2810) μg/L	Adult (24 h) Embryo (3 h)	↑ (GstPi, GCLC, HO-1, Prdx1, Nrf2)	[87]
Cadmium	C57BL/6 mice	2 and 7 mg/kg	4 and 8 weeks	↑ (NLRP3, Asc, Cas 1, Gsdmd, IL-1β, and IL-18, Keap 1), ↓ (Nrf2)	[88]
Cadmium	*Swiss* albino mice	6.5 mg/kg	7 days	↓ (LH, FSH, GPx, SOD, CAT, GR, Nrf2, Bcl2), ↑ (IL-1β, TNFα, Cas 3, Bax)	[89]
Cobalt	*Wistar* rats	50 mM	Once/10 days	↑ (HIF1α, NOX2), ↓ (Nrf2)	[96]
Cobalt	*Wistar* rats	4 mg/kg	20 days	↑ (MDA, Cas 9), ↓ (Nrf2, HO-1)	[97]
Lead	*Sprague* Dawley rats	200 ppm	8 weeks	↑ (Bax/Bcl2), ↓ (Nrf2, CAT, Mn-SOD)	[99]
Lead	*Wistar* albino rats	20 mg/kg	7 days	↑ (Pb accumulation, LPO, NO, IL-1β, TNFα, iNOS, Bax, Cas3), ↓ (SOD, CAT, GSH, GPx, GR, Nrf2, HO-1, Bcl2)	[101]
Lead	*Wistar* rats	20 mg/kg	7 days	↓ (SOD, CAT, GPx, GR, Nrf2, HO-1, Bcl2), ↑ (Pb accumulation, MDA, TNF-α, IL-1β, NO, Bax, Cas 3)	[52]
Lead	ICR mice	250 mg/L	4 weeks	↑ (MDA, Cas 3, Cyt C, TNFα, COX2, NF-kβ), ↓ (SOD, TAC, Nrf2, HO-1, NQO1, Bcl2)	[102]

ALP- Alkaline phosphatase.
LDH- lactate dehydrogenase.
ICR- Institute of Cancer Research.
Ucp2-Uncoupling protein 2.
AChE- Acetylcholinesterase.
BChE- Butyrylcholinesterase.
mTOR- Mammalian target of rapamycin.
MPO- Myeloperoxidase.
Asc - Apoptosis-associated speck-like protein.
Gsdmd - Cleaves gasdermin D.

Several studies showed that cobaltous chloride ($CoCl_2$) induced hypoxia caused neurotoxicity in both *in vivo* and *in vitro* models. Administration of $CoCl_2$ increased ROS, mitochondrial dysfunctioning, and apoptotic cascade together with decrease in Nrf2 and increase in HIF-1α, thus disrupting Nrf2-GCL regulated homeostasis [95,96]. Another study showed significant brain damage in the cortex and hippocampal regions following exposure to cobalt nanoparticles (CoNPs) and $CoCl_2$. Additionally, there was a rise in MDA content, Cas 9 protein level, and HO-1 in experimental rats. Decreased cell survival and a higher rate of apoptosis were the results of Nrf2 downregulation in PC12 cells. In this way, Nrf2 acted as a shield for brain cells against the damaging effects of $CoCl_2$ and CoNPs by pushing up antioxidant responses [97]. According to an *in vitro* study, the Nrf2 signaling pathway may guard human keratinocytes (HaCaT) from acute $CoCl_2$-induced hypoxia cytotoxicity by participating in the antioxidant response brought on by oxidative stress generated by $CoCl_2$. It elevated ROS significantly, causing hypoxic injury and HaCaT cell cytotoxicity. Numerous antioxidant enzymes were drastically downregulated upon stable knockdown of Nrf2, and the cells became more susceptible to acute $CoCl_2$-induced oxidative stress and cytotoxicity. On the other hand, Nrf2 and ARE-regulated gene activity was clearly increased in Keap1-KD cells, which resulted in a notable resistance to cellular damage caused by $CoCl_2$ [98].

Lead (Pb) - It is a hazardous element that is frequently used in industry. It can be found in a variety of products, including paints, toys, cosmetics, shot ammunition, fishing weights, and Pb-acid batteries, as well as in the air, dust, food, and drinking water [85]. Pb is known to be an environmental contaminant that, even at low concentrations, poses a risk to human health and can result in serious immunological, neurological, gastrointestinal, cardiovascular, and reproductive problems [85]. Studies conducted *in vivo* and *in vitro* have validated that exposure to Pb can be connected with a decline in the antioxidant activity of several enzymes and; additionally, scientists have proposed a possible correlation between Pb exposure and Nrf2 signaling [99,100].

A study in recent past showed that 20 mg/kg lead acetate (PbAc) revealed suppressed sex hormone levels and an upsurge in Pb concentration in testicular tissue. PbAc decreased the activity of CAT, SOD, and GSH and deactivated HO-1 and Nrf2 at the molecular level. Additionally, testicular tissue exposed to PbAc toxicity displayed a decrease in Bcl-2 and an increase in inflammatory and apoptotic cascades [101]. Additionally, Albarakati et al., reported that Pb treatment resulted in elevated MDA levels and decreased antioxidant enzyme activity and expression of SOD, CAT, GSH-Px, and GR in rat kidneys. In the same study, exposure to Pb increased the levels of TNF-α, IL-1β, and NO in kidney, while downregulated the expression of Nrf2 and HO-1 mRNA [52]. Furthermore, the inhibition of Nrf2 and HO-1 stimulation along with a reduced nuclear translocation of Nrf2 in the mouse brain may be related to oxidative damage, inflammation, and neuronal degeneration in hippocampal tissue taken from mice after a 4-week Pb administration [102].

Pb exposure in bovine granulosa cells caused oxidative stress, decreased proliferation of cells, changed cell cycle progression, and eventually resulted in apoptosis by interfering with the Nrf2/NF-κB connection. There was a considerable downregulation of both SOD and CAT levels, along with a substantial decrease in the levels of Keap1 mRNA, while Nrf2 exhibited a large decrease [100]. Microglia subjected to lead-induced oxidative stress produce more ROS, down-regulate GSH, and express the Nrf2 protein more prominently than astrocytes [103]. In SH-SY5Y cells, PbAc mostly displayed neurotoxicity through oxidant-based mechanisms. Cells exposed to PbAc triggered a defense response that increased Nrf2 nuclear accumulation and, in a ROS-dependent way, Nrf2-ARE binding activity. PbAc activated the mRNA expression of HO-1, GSTα1, GCLC, and NQO1, as well as the protein expression of HO-1 and γ-glutamylcysteine synthetase (γ-GCS) [104]. Similarly, SH-SY5Y cells exposed to different concentrations of PbAc increased the expression of Bax, Cyt C, and Cas 3 together with the lowered expression of Bcl-2 [105].

Summary of the effects of various metals/metalloids *in vitro* and *in vivo* models on Nrf2 signaling and its interrelation with oxidative stress parameters are given in Tables 1 and 2 respectively.

1.3. Therapeutic implications of Nrf2 signaling in heavy metals-induced oxidative stress

The human biological system's exposure to heavy metals causes oxidative stress, which further impairs protein function, damages DNA, and causes lipid peroxidation [110]. Chelation therapy is a recognised, effective treatment for heavy metals poisoning. However, despite the chelating drugs' proven efficacy, several severe side effects can happen [111]. Conventional chelating agents such as calcium disodium ethylene diamine tetra acetic acid ($CaNa_2EDTA$), meso 2, 3-dimercaptosuccinic acid (DMSA), and British anti-lewisite (BAL), to cite a few, are far from being the perfect chelator because of their high toxicity and frequent occurrence of different adverse effects. For instance, BAL increased the toxicity of Cd and Pb in animal tests, and reports of increased brain deposits for As^{+3} and organic Hg compounds have been made in response to BAL administration [112].

Using two chelators that have different actions is a recent approach in chelation therapy. Combining treatments is predicated on the theory that different chelating agents will likely mobilise harmful metals from distinct tissue compartments, potentially leading to better outcomes. Compared to other treatments, the co-administration of DMSA and MiADMSA at a lower dose (0.15 mmol/kg) was most efficient in lowering arsenic-induced oxidative stress as well as removing arsenic from blood and soft tissues. This combination also demonstrated significant effectiveness in repairing DNA damage caused by arsenic exposure [113]. Combinatorial medication delivery has recently demonstrated synergistic therapeutic effects against As-generated oxidative damage approach using nano-Mi monoisoamyl (ADMSA) and nano-curcumin in rats [114].

Nrf2 is a therapeutic target that shows potential in the development of treatment options to counteract oxidative stress caused by heavy metals. Several *in vivo* and *in vitro* studies suggest that natural substances and synthetic small compounds such as dimethyl fumarate [115] and bardoxolone methyl [116] can activate the Nrf2 antioxidant pathway and provide protection against oxidative damage in neurodegenerative diseases and acute kidney injury respectively.

Over the past ten years, dietary components have demonstrated their capacity to lessen the toxicity of environmental pollutants. Additionally, an increasing amount of scientific research suggests that phyto-antioxidants have a role in mitigating or preventing the

harm caused by heavy metals because of their antioxidant nature, metal-chelating qualities, and modification in the regulation of defense genes through the Nrf2/ARE pathway [56,117]. These naturally occurring antioxidants include lutein, lycopene, flavonoids, phenolic compounds, isoflavones, carotenoids, and tocopherols. They can scavenge free radicals, prevent oxidation, and serve as chelators and reductants. Transition metal ions may be chelated by many flavonoids. This is because the many hydroxyl groups can combine with metals to produce complexes that hinder their absorption through the gastrointestinal tract and hasten their excretion in urine [118]. Additionally, polyphenols have the ability to stop the activity of enzymes involved in the synthesis of reactive oxygen forms [111]. The role of flavonoids as antioxidants has received much attention lately. Recently, authors investigated quercetin's ability to protect rats from Cd-induced cognitive impairments by modulating the PI3K/AKT—Nrf2/ARE signaling pathways in the hippocampus and N-Methyl-D-aspartate receptor (NMDA)-R-mediated downstream signaling [119]. Methionine and cysteine, together with N-acetylcysteine, an acetylated version of cysteine, S-adenosylmethionine, a metabolite of methionine, α-lipoic acid, and the tripeptide GSH, are polysaccharides implicated in the elimination of heavy metals. By forming a stable complex and protecting biological targets from metal ions, these chemicals efficiently bond with toxic heavy metals, reducing their negative effects and triggering their excretion from the body [120].

The effects of the 28-day quercetin treatment (25 mg/kg b.w.) on hippocampal alterations caused by Cd were mitigated [119]. Specifically, the treatment led to a reduction in oxidative stress markers and an improvement in synaptic plasticity. Curcumin, on the other hand, is a polyphenolic molecule that has a good chelating activity for metal ions due to the reactivity of its α-β-unsaturated

Table 3
Summary of the therapeutic implications of Nrf2 signaling in heavy metals -induced oxidative stress.

S. No.	Environmental toxicant and experimental model	Treatment and Dose	Duration	Results	Reference
1.	Chromium exposed mice	CoQ10- 10 mg/kg, BCA- 50 mg/kg, PHL- 50 mg/kg	30 days	↓ (PCC, MDA), ↑ (GSH, SOD, GST, TT, CAT, Nrf2, HO-1, NQO1)	[56]
2.	Cadmium exposed rats	Quercetin-25 mg/kg	28 days	Modulation in Nrf2/ARE signaling resulting in controlled NMDA-R and PI3K/AKT cell signaling.	[119]
3.	Arsenic treatment in PC12 cells	Curcumin (2.5 μM)	Pretreatment	↑ (mTOR, Akt, Nrf2, ERK1, Bcl-x) ↓ (ULK, LC3, p53, Bax, Cyt C, Cas 9, cleaved Cas 3)	[122]
4.	Chromium and Arsenic exposed mice	CoQ10- 10 mg/kg, BCA- 50 mg/kg, PHL- 50 mg/kg	15 days	↓ (PCC, MDA), ↑ (GSH, SOD, GST, TT, Nrf2, AChE, BChE)	[55]
5.	Arsenic exposed mice	BCA-50 mg/kg, PHL-50 mg/kg, EGCG-40 mg/kg	2 weeks	↓ (PCC, MDA), ↑ (GSH, SOD, GST, TT, Nrf2)	[54]
6.	Lead exposed mice	Ferulic acid-50 mg/kg	31 days	↓ (MDA), ↑ (SOD, GSH, glutamate cysteine ligase, Nrf2, HO-1)	[124]
7.	Silver nanoparticles exposed mice	Naringenin- 25, 50, 100 mg/kg	3 days	↓ (WBC, Neutrophil, lymphocyte), ↑ (Nrf2, HO-1)	[125]
8.	Silver nanoparticles exposed Human lung epithelial BEAS-2B cells	Naringenin- 25, 50, 100 μM	Post-treatment	↓ (Bax, CytC, cleaved Cas 9, cleaved Cas 3) ↑ (Bcl2, Nrf2, HO-1)	[125]
9.	Lead exposed mice	Mangiferin-50, 100, or 200 mg/kg	4 weeks	↑ (Nrf2, HO-1, NQO1, γ-GCS)	[126]
10.	Arsenic trioxide exposed mice	Hesperidin-100, 300 mg/kg	1 week	↓ (CK, LDH, cTnI, ROS, MDA, TNF-α, IL-6, Bax, Cas 9, cleaved Cas 3, Keap1) ↑ (SOD, GSH, CAT, Bcl-2, p62 and Nrf2)	[127]
11.	Lead exposed mice	Cordyceps militaris (CEP-I)-50, 100, or 200 mg/kg	4 weeks	↓ (MDA), ↑ (SOD, Nrf2, HO-1, NQO1, Keap 1)	[129]
12.	Cadmium exposed TM3 cells	Sulforaphane- 0, 1.25, 5, 10, 20, 40, and 80 μmol/L	24 h	↓ (MDA, ROS), ↑ (T-SOD, GSH-Px, GSH, Nrf2, GSH-Px, HO-1, NQO1, γ-GCS)	[131]
13.	Cadmium exposed mice	Sulforaphane-10 mg/kg	10 days	↓ (MDA), ↑ (T-SOD, GSH, Nrf2, GSH-Px, HO-1, NQO1, γ-GCS)	[132]
14.	Cadmium exposed rats	Vitamin E−100 mg/kg	4 weeks	↓ (ALT, AST, accumulation of Cd) ↑ (Nrf2, HO-1, NQO-1, GCLC, GCLM and GST)	[133]
15.	Lead exposed rats	Vitamin D-1000 IU/kg	4 weeks	↓ (MDA, ROS), ↑ (SOD, GSH, CAT, Nrf2)	[134]
16.	Arsenic trioxide exposed Chang liver cells	Ascorbic acid-100 μM αTocopherol-50 μM	48 h	↓ (MDA), ↑ (Nrf2, Bcl2)	[72]

CK- creatine kinase.
LDH- lactate dehydrogenase.
cTnI- cardiac troponin I.
8-OHdG- 8 hydroxy-2′-deoxyguanosine.
ULK- unc-51-like kinase 1.
LC3- Microtubule-associated protein 1A/1B-light chain 3.

β-diketone moiety, which strongly coordinates several different metal ions, including as Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} [121]. Curcumin also exhibited an antioxidant effect by controlling the Nrf2 antioxidant signaling system by decreasing the As toxicity in PC12 cells by regulating autophagy and apoptosis [122]. Authors have reported that curcumin can up-regulate the (Keap1)/Nrf2/ARE pathway, which in turn induces detoxifying enzymes along with the downregulation of NF-κB, as well as the expression and concentration of pro-inflammatory cytokines, by preventing the damaging effects of heavy metals on the liver [123]. Studies in the past have also reported that various nutraceuticals viz., biochanin-A, phloretin, coenzyme Q10, and epigallocatechin gallate were found to be effective in mitigating Cr and As induced oxidative stress as singlet and in combination via upregulating the Nrf2/HO-1/NQO1 signaling pathway in different tissues of experimental models [54–56]. Furthermore, Yu et al. [124] demonstrated that ferulic acid plays a crucial role in activating the Nrf2-mediated antioxidant defense mechanism in mice, thereby preventing Pb-induced cognitive deficits. The administration of ferulic acid to newborn mice has even been found to protect their offspring from Pb-induced cognitive damage, highlighting its potential as a protective agent. These results support the theory that ferulic acid enhance Nrf2 and associated signaling pathway, and has a significant protective impact on the brain against Pb-induced neurotoxicity. Naringenin mitigated silver nanoparticle (AgNP)- induced oxidative stress and inflammatory response. It also lowered AgNP-induced apoptosis by modifying low levels of Bax, Cyt C, cleaved Cas 9, and cleaved Cas 3 but elevated Bcl2. Additionally, it successfully reduced ferroptotic indicators, elevated HO-1 and Nrf2 protein expressions, and boosted Nrf2's nuclear translocation [125]. After eight weeks of exposure to Pb-containing water, the brain developed pathological changes and anti-oxidative system dysfunction, which were all prevented by mangiferin in an Nrf2-dependent way. Mangiferin was found to stimulate Nrf2 downstream enzymes, including HO-1, NQO1, and γ-GCS, and significantly reduce morphological damage in the hippocampal region. These findings highlight the potential of mangiferin as a powerful tool in mitigating the harmful health effects associated with Pb exposure [126]. In animal models of As cardiotoxicity, hepatotoxicity, and nephrotoxicity, hesperidin advantageously regulates oxidative stress, inflammation, p53 protein, p62/Keap1/Nrf2 signaling, and DNA damage [127,128]. As reported in a past study, polysaccharides play a significant role in regulating the Nrf2/Keap1 pathway. Authors found that Cordyceps militaris prevented Pb^{2+}-induced liver and kidney toxicity by activating Nrf2/HO-1/NQO1 antioxidant pathway and modulating gut microbiota [129]. An organic sulphur component called sulforaphane (SFN) is obtained from crucifers like olives, broccoli, and cabbage. Due to its remarkable ability to drastically minimize tissue or structural damages induced by oxidative stress, it is one of the naturally active antioxidants with the highest efficiency [130]. In an *in vitro* study, authors have demonstrated that upon SFN treatment, the mRNA expression of Nrf2 and associated genes in Cd-treated cells gets upregulated. This reveals the protective effects of SFN against Cd-induced oxidative stress through activation of the Nrf2/ARE signaling pathway [131]. Additionally, Yang et al. (2016) demonstrated that SFN dramatically decreased testicular cell death and increased Nrf2, HO-1, and NQO1 expression levels in *Kunming* mice [132]. It has also been concluded that several vitamins, such as vitamin E [133], vitamin D3 [134], vitamin C [72], have beneficial effects in combating heavy metal-induced toxicity in various experimental models by mitigating the induced oxidative stress via activating the Nrf2 antioxidant signaling pathway. These findings offer a promising avenue for further research and potential solutions.

A summary of the therapeutic implications of Nrf2 signaling in heavy metals -induced oxidative stress are given in Table 3.

2. Conclusion and future prospects

Nrf2, a key regulator of oxidant resistance, was first identified over a decade ago as the transcription factor responsible for the induction of ARE-dependent drug-metabolizing enzymes. Since then, it has been associated with various toxicities and chronic diseases linked to oxidative damage. By controlling oxidant levels and signaling, Nrf2 modulates multiple programmed processes, including autophagy, inflammasome signaling, UPR, apoptosis, mitochondrial biogenesis, and stem cell regulation. Numerous studies have emphasized how it is related to toxicity caused by metals and metalloids. While some findings suggest that exposure to toxic metals or metalloids increases Nrf2 activation through pathways dependent on Keap1, protecting against their toxicity, other findings suggest that such exposure inhibits the Nrf2-ARE defense pathway. Several studies have implicated disruption of Nrf2 signaling pathways in the pathogenesis of heavy metal-induced cancer and neurodegeneration. Therefore, the development of a potent prophylaxis or therapeutic that can fortify the Nrf2-dependent adaptive mechanism and protect individuals against contaminants like metalloids and toxic metals is not just necessary; it is paramount in a current scenario. A growing body of research indicates that dietary nutraceuticals and small molecules of Nrf2 activators administered individually or in combination have a variety of pleiotropic benefits, including protection against metal-induced toxicity, reduction of inflammatory damage, cancer prevention, preservation of lipid metabolism, and reduced oxidative stress. It is now acknowledged that antioxidant gene inducers directly target Keap1. However, the precise mechanisms of how particular nutraceuticals interact with the Keap1 protein are still unknown. Thus, a thorough knowledge of Keap1 reactivity and an understanding of the control of Nrf2 activity will aid in creating an effective Nrf2-targeted therapy design. Future research should focus on understanding the structural and chemical characteristics of inducers of antioxidant genes. Also, studies must focus on finding novel natural and synthetic Nrf2 activators with enhanced efficacy, safety profiles, and specificity to combat metal-induced toxicities effectively. The synergistic role of antioxidants and metal chelators to improve therapeutic outcomes and total antioxidant defenses can be explored further. As a significant step in determining the safety and efficacy of dietary nutraceuticals and small molecules in the human population and accelerating their development into therapeutic interventions for disorders linked to heavy metal exposure, the progress toward carefully designed clinical studies will be evident. These future directions, with their urgency and potential, will help to create novel therapeutics to mitigate heavy metal-induced toxicities and manifest the potential of Nrf2 signaling pathways into clinical translations.

Data availability statement

Being a review article, data sharing does not apply as data was analyzed from the published studies.

CRediT authorship contribution statement

Swapnil Tripathi: Writing – original draft, Investigation, Data curation. **Gitika Kharkwal:** Methodology, Data curation. **Rajeev Mishra:** Writing – review & editing, Data curation. **Gyanendra Singh:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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