Review Article

An Update on the Role of Nrf2 and its Activators in Diseases Associated with Oxidative Stress

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A wide range of experimental and observational studies have established the irrefutable role of oxidative stress in the pathogenesis of a plethora of diseases either directly or indirectly. Several factors ranging from radiation, pollution, high fat and sugar diet, alcohol consumption, smoking, tobacco consumption and even certain drugs contribute to oxidative stress. The imbalance between oxidant and antioxidant levels remain the underlying cause of oxidative stress. When the levels of oxidants outweigh the levels of antioxidants it leads to formation of free radicals like hydroxyl, superoxide, alkoxyl, peroxyl, glutathiyl, tocopheroxyl, hydroperoxyl and ascorbate. These free radical species play a definitive role in the pathogenesis of neurological diseases, malignancies, cardiovascular, respiratory and liver diseases. Nuclear factor erythroid-2-related factor 2 is a master transcription factor belonging to the leucine zipper family. Kelch-like ECH-associated protein 1 is a repressor of nuclear factor erythroid-2-related factor 2 under basal or normal conditions, which is responsible for cytoplasmic sequestration and proteosomal degradation of nuclear factor erythroid-2-related factor 2 via ubiquitination. The activation of nuclear factor erythroid-2-related factor 2/Kelch-like ECH-associated protein 1/antioxidant response element signalling pathway regulates the expression of numbers of genes that are cytoprotective, antioxidative and detoxificative in action. This article reviews the potential therapeutic role of nuclear factor erythroid-2-related factor 2 activators in prevention and treatment of those diseases in which oxidative stress plays a definitive role in the pathogenesis.

Key words: Nuclear factor erythroid-2-related factor 2, keap1, antioxidant response element, oxidative stress, free radicals

Oxidative stress (OS) plays a major role in the development and progression of a wide range of diseases including Alzheimer disease, cancer, atherosclerosis[1], chronic kidney disease[2], multiple sclerosis, Niemann-Pick C, Parkinson's disease, Friedreich's ataxia, Huntington's disease (HD), infantile neuroaxonal degeneration, neurodegeneration with brain iron accumulation (NBIA) and lipid metabolism deregulation syndrome like Zellweger syndrome[3]. An assumption also exists currently that OS could cause cancer in patients with Type 2 Diabetes Mellitus (T2DM)[4,5]. The neurons and oligodendrocytes of the central nervous system are particularly more susceptible to OS[6]. Reactive Oxygen Species (ROS) include free radicals such as superoxide ($^{\bullet}O_2^-$), peroxyl ($^{\bullet}RO_2$), hydroxyl ($^{\bullet}OH$), hydroperoxyl (HRO_2), and nonradical species such as hydrochlorous acid (HOCl) and hydrogen peroxide (H_2O_2)[7]. ROS are the small oxygen-containing end-products of aerobic metabolism, which are highly reactive[8]. The increase in the level of these species often disrupt the normal oxidative-redox system[9]. In the recent times several works have reported the relationship between the nuclear factor E2-related factor 2 (Nrf2) and OS[10-13]. Nrf2, a nuclear transcriptional factor is observed to show a protective role in cell defence and survival against xenobiotics and OS[14] via the control and regulation of the production of number of genes and enzymes that function as antioxidants. Some of the antioxidant enzymes produced through the activation of Nrf2 are, superoxide dismutase (SOD), sulfiredoxin (Srx), peroxiredoxin (Prx), GSH peroxidase (GPx) and sestrin2 (Sesn2), which are involved in catabolism of

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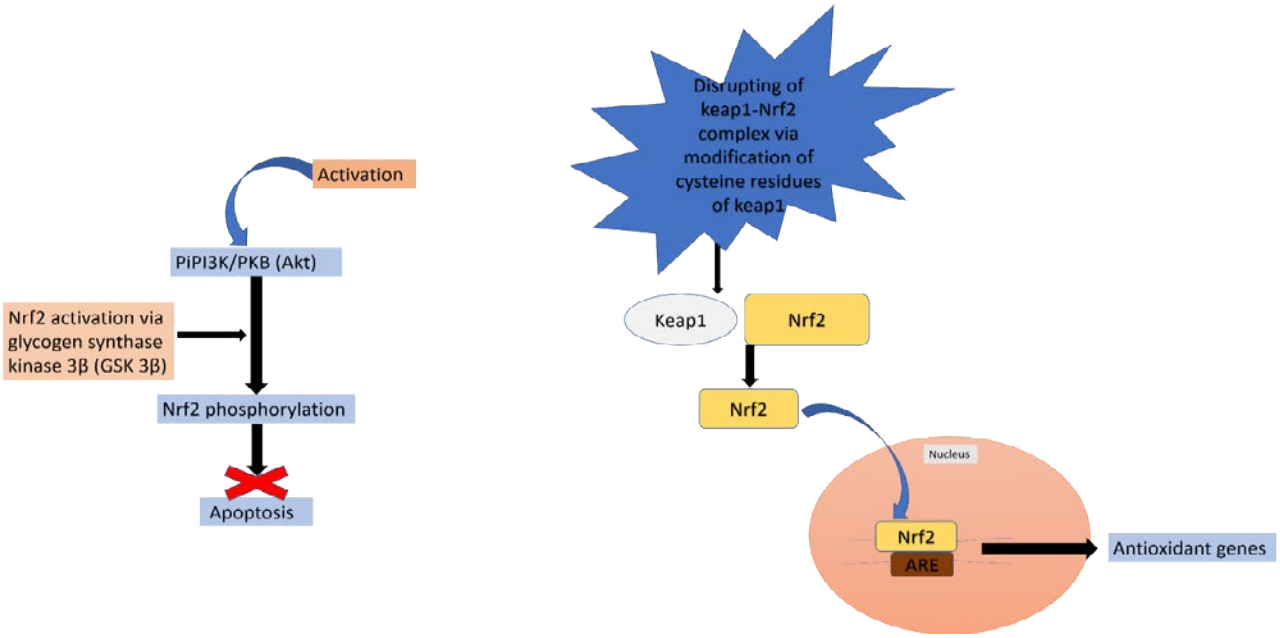

Fig. 1: Mechanism of Nrf2 activators

free radicals into hydrogen peroxide, which is further broken down into water and oxygen by the enzyme catalase. Nrf2 also regulates the synthesis of GSH with glutamate-cysteine ligase modifier (GCLM) subunit and glutamate-cysteine ligase catalytic subunit (GCLC). Nrf2 also increases the redox cysteine/ glutamate transport and peroxidase, which is a hemoprotein that uses hydrogen peroxide to oxidize a number of substrates. Nrf2 also regulates the levels of catalase, a major defence enzyme against OS which is responsible for conversion of hydrogen peroxide into water and oxygen, and NADPH production by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD). Nrf2 also augments the activity of stress response proteins, such as heme oxygenase-1 (HO-1), which affects a variety of cellular functions and upregulation of metal chelators, metallothionein1/2 (MT1/2) and ferritin. Non-enzymatic antioxidants like NAD(P)H quinone oxoreductase (NQO1), GPx, glutathione S-transferase, and thiols are also upregulated by Nrf2[2,15,16]. It is clear that Nrf2 plays a role in protecting cells against OS and this property of Nrf2 makes it an interesting target in several diseases where OS plays a significant role in pathogenesis.

Overview of NRF2- Kelch-like ECH-associated protein 1 (Keap1):

Nrf2 is a master transcription factor belonging to the leucine zipper family[17]. It contains a basic leucine-zipper (bZip) domain at the C-terminus and participates in the formation of heterodimers with other bZip proteins like muscle aponeurosis fibromatosis (MAF)[18]. Under normal conditions Keap1, a cytoplasmic protein associated with the Cullin3 (Cul3) based E3 ligase complex, binds to Nrf2 leading to proteosomal degradation of Nrf2 via ubquitination[19,20]. Nrf2 consists of 6 highly conserved homology domains named Neh1 to Neh6 (Nrf2-ECH homology). Among all, in fibroblasts, Neh2 is a dependent transactivation domain and Neh4 and Neh5 are 2 independent activation domains[21]. Keap1 and β-transducin repeats-containing proteins (β-TrCP) are E3 ubiquitin ligase adaptor proteins that regulates Nrf2 intracellularly by association with its two degradation domains, Neh2 and Neh6[22]. Cytoplasmic localization of Nrf2 is determined by Neh2, recruitment of transcription factors and other canonical protein needed for gene expression are determined by Neh4 and Neh5. Kelch domain and the bric-a-brac, tram track, broad-complex (BTB) domain are the two domains of Keap1. Kelch binds to actin thereby tie the Keap1-Nrf2 complex to the cytoskeleton and BTB is important for Keap1-protein dimerization[23]. The conformational modifications by ectopic and endogenous electrophiles (residues C151, C273 and C288) from the sulfhydryl groups of Keap1 allow Nrf2 to escape Keap1-dependent degradation. Thus, Nrf2 accumulates in the nucleus and activates ARE-genes. On the other hand, β-TrCP, which is a homodimeric E3 ligase adapter also signals Nrf2 modification via phosphorylation by glycogen synthase kinase-3 (GSK-3) that promotes proteasomal degradation. Therefore, GSK-3/β-TrCP also serves as a potential target to increase the activity of Nrf2[24,25]

Mechanism of action of nrf2 activators:

Several mechanisms have been proposed for activation of Nrf2 at the molecular level. One hypothesis is that activation of PI3K/PKB (Akt) signalling leads to phosphorylation of Nrf2 by glycogen synthase kinase 3β. Akt, a downstream target of PI3K prevents cells form undergoing apoptosis. Another hypothesis is that the degradation of Nrf2 is regulated by a protein called Keap1. The binding of keap1 to Nrf2 leads to proteasomal degradation of Nrf2. Disruption of this complex via modification of cysteine residues within the Keap1 destabilizes the Nrf2–Keap1 complex thereby allowing the free translocation and nuclear accumulation of Nrf2. Nrf2 once inside the nucleus binds to ARE and activates the transcription of cytoprotective genes[26-29].

Diseases-related OS:

An overproduction of ROS and the deficiency in the levels of antioxidants leads to OS (fig. 2)[30]. In alcohol abuse, there is a surge in OS markers and increase in lipid peroxidation products (F2-isoprostane and 4-hydroxy-2-nonenal) but depletion of antioxidants like glutathione (GSH) and vitamins E and C (fig. 3). In hepatitis, hepatocellular and cholestatic dysfunction, DNA damage, lipid damage and mitochondrial dysfunction occurs as a result of OS (fig. 4). In renal disease like CKD, end-stage renal disease and chronic inflammation, there is an impairment of activation of Nrf2 and diminished antioxidant defence (fig 4)[31]. In T2DM the Nrf2 induction suppressed the formation of DNA adducts via ROS-induction, intracellular ROS formation, apoptosis of pancreatic β-cells within the islets as well as a reduction in inflammation via NFκB pathways. Fig. 5 denotes the role of ROS and the effects of Nrf2 activation in regulation of OS in T2DM[32]. ROS has also been reported to increase apoptosis in Sertoli cells by decreasing the expression of blood-testis barrier (occludin, N-cadherin, zonula occludens-1 and β-catenin) and disordering the F-actin spatial arrangement. This is believed to be because of the upregulation of Jun N-terminal (JNK), extracellular signal regulatory kinase (ERK), p38 mitogen-activated protein kinase (MAPK) and downregulation of Nrf2. This, indicates that ROS-MAPK-Nrf2 is involved in spermatogenesis dysfunction[33]. A similar study on prepubertal testicular injury has suggested the role of OS increase through inhibition of Nrf2-mediated antioxidant signalling pathway[34].

In AD, OS and cytotoxicity via the alteration of mitochondrial membrane potential by amyloid beta (Aβ), and the complex of Aβ42 and copper (I) ion leads to reduced oxygen level and generation of H_2O_2. Interestingly, the study of AD using *Drosophila* model showed that there was a decrease in the level of Aβ42 peptide levels upon down regulation of Keap1, suggesting that reducing the activity of Keap1 may clear Aβ42 peptide via protein degradation. In PD, aggregation of α-synuclein protein, loss of function of parkin protein and mutation of DJ-1 are known to play a major role in the generation of OS[35,36]. In case of HD, OS results due to the effect of mutant huntingtin (mHtt) that disrupts the Nrf2 signalling pathway[37]. Examples of agents that help in combating OS via the activation of Nrf2 by persulfidation of Keap1 are S-1-propenylmercaptocysteine (CySSPe) and N-acetylcysteine. CySSPe was reported to increase hydrogen sulfide (H_2S) production that persulfidates keap1, enhance the cellular production of GSH and improve GSH:GSSG ratio[38]. N-acetylcysteine is

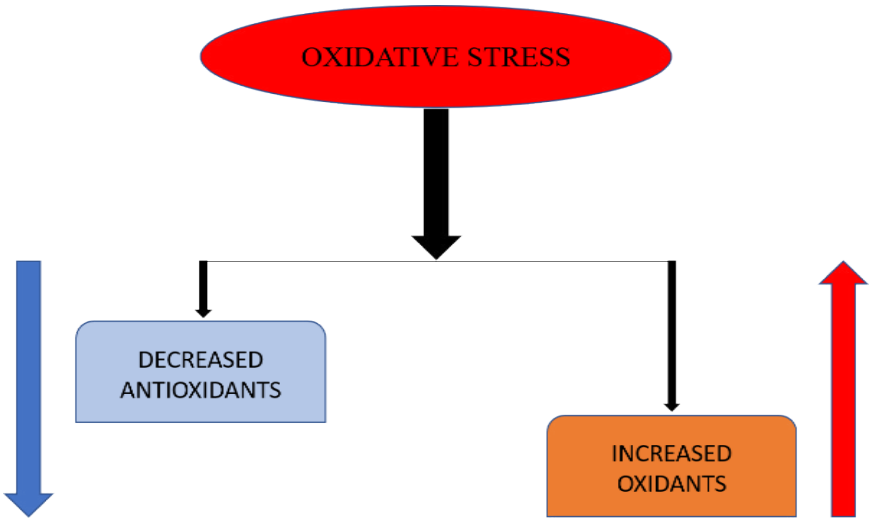

Fig. 2: Oxidative stress due to decreased antioxidants and increased oxidants

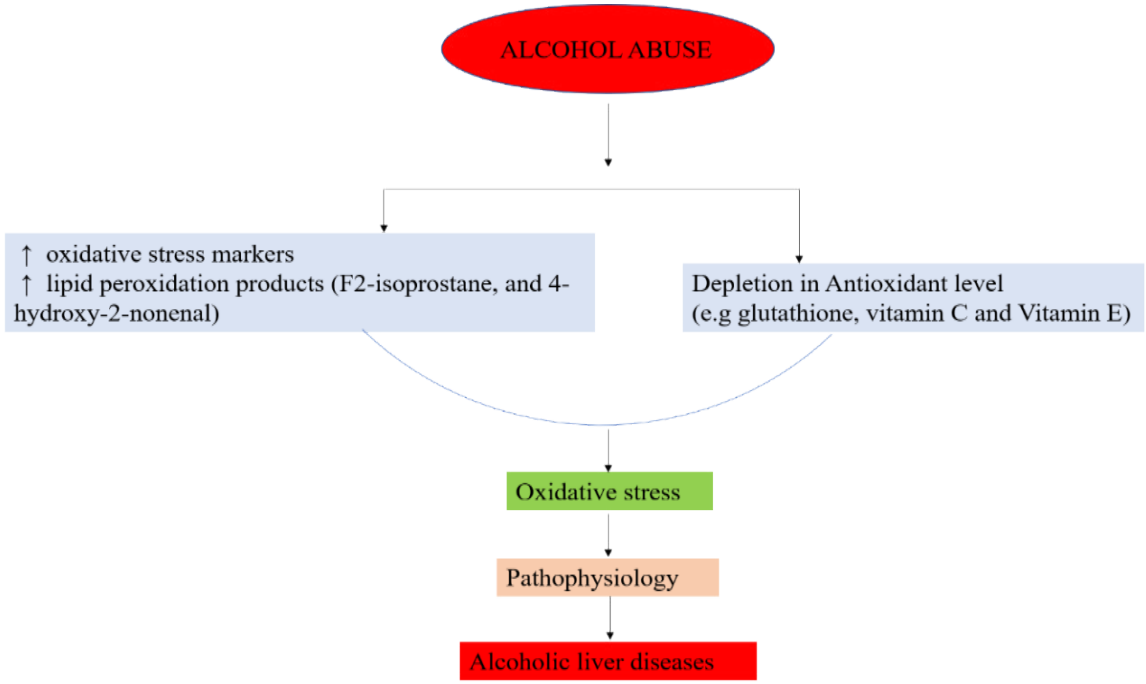

FIG. 3: The increased and decreased in bio markers in alcohol abuse leading to oxidative stress

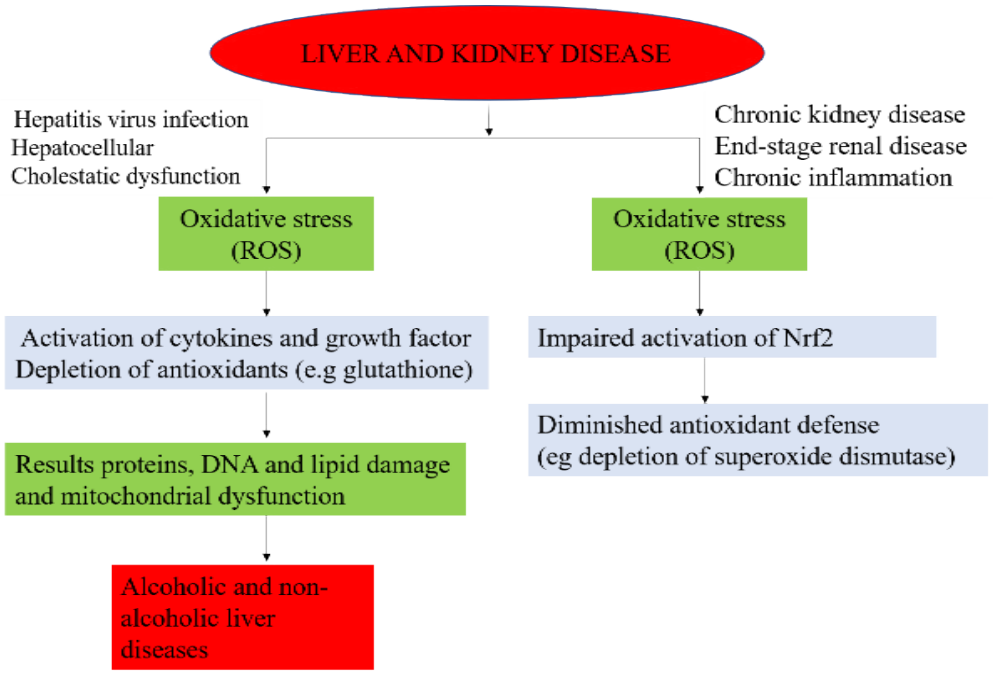

Fig. 4: Impairment of liver and kidney function as a result of oxidative stress
Impairment of liver function in hepatitis, hepatocellular and cholestatic dysfunction and kidney function during CKD and end-stage renal disease and chronic inflammation as a result of oxidative stress

a pro-drug that is widely used as an antidote against acetaminophen overdose. It is a cysteine derivative and a direct precursor of GSH with beneficial effects in OS-related diseases caused by pesticides[39].

NRF2 activators:

The current consensus is that Nrf2 activators stabilize and promote Nrf2 translocation by inhibiting Keap1 from binding to Nrf2. Nrf2, on translocation into the nucleus leads to production of antioxidant and antiinflammatory substances that decrease OS and inflammation[40]. A few examples of Nrf2 activators are oleanolic acids (OAs), bardoxolone methyl, dimethyl fumarate (DMF) and sulforaphane (SFN), calastrol, ellagic acid, zerumbone, curcumin, quercetin, quercitrin, resveratrol, phenethyl isothiocyante (PEITC), lucidone, forsythoside B and thymoquinone. Fig. 6 represents the chemopreventive/chemoprotective natural products

and their effects upon Nrf2 activation[41,42]. Dimethyl fumarate (DMF) has been reported to activate Nrf2 and increase its activity in neuronal subpopulations but not in astrocytic cells[43,44]. The activation of Nrf2 by a bioactive compound, salvianolic acid B (SalB) has been reported to protect dopaminergic neurons in PD via the

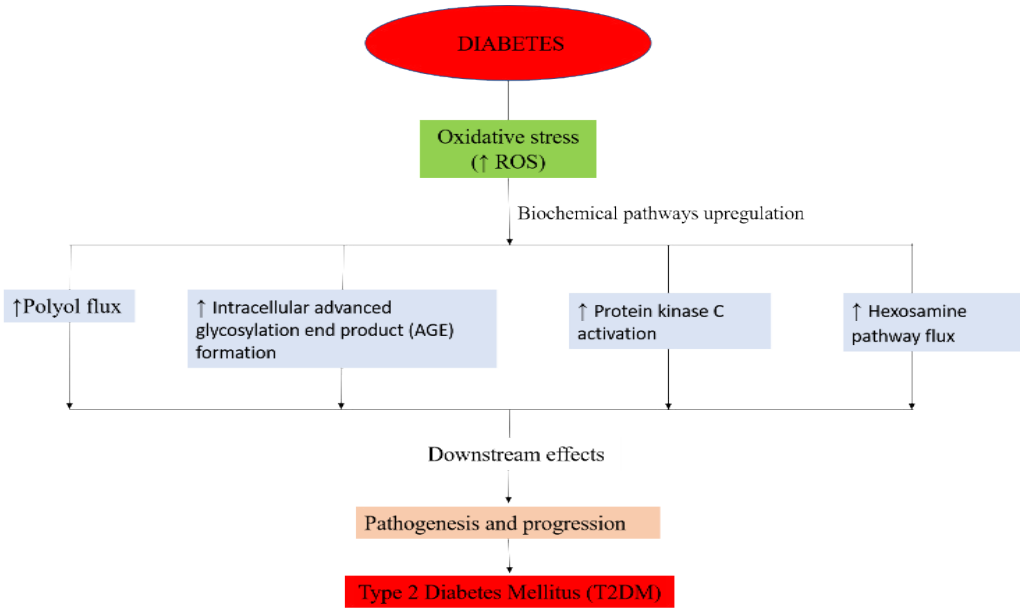

Fig. 5: Role of increased oxidative stress in T2DM

Fig. 6: Chemopreventive/chemoprotective natural products and their effects upon Nrf2 activation

inactivation of microglia-mediated inflammation and through induction of astrocyte activation-dependent GDNF expression[45]. Similarly, in a rat PD model with severe OS induced by 6-hydroxydopamine (6-OHDA) pre-treatment with phloroglucinol, a polyphenol salvaged the decrease in Nrf2 and p-Nrf2 in the nuclear fraction[46]. In a similar study in a rat PD model with OHDA as an inducing agent, icariin targets Nrf2 signalling by inhibition of microglia-mediated neuroinflammation[47]. Nrf2 plays an vital role in maintaining newly generated hepatocytes during liver repair[48,49]. Activation of Nrf2 by pre-treatment with resveratrol in mice heart has been reported to alleviate the levels of mitochondrial ROS production induced by lipopolysaccharide in cardiomyocytes[50]. In another study, pre-incubation of Sertoli cells with luteolin, a natural flavonoid, induced the expression of antioxidant enzymes and increased ARE-luciferase reporter activity via triggering Nrf2 translocation[51]. In case of mice with transverse aortic arch constriction (TAC) model with maladaptive cardiac remodelling and dysfunction, dihydro-CDDO-trifluoroethyl amide (dh404) up-regulates myocardial Nrf2 protein expression and inhibit cardiomyocyte hypertrophy and proliferation of cardiac fibroblast in chronic pressure overload, heart failure and maladaptive cardiac remodelling and dysfunction[52]. Sulforaphane, the most abundant isothiocyanate has been reported to alleviate or retard the ischemia-reperfusion induced retinal damage and grape seed proanthocyanidin has been shown to have a protective role in diabetic bladder dysfunction via the activation of Nrf2/HO-1 pathway[53,54]. Sulforaphane has also been reported to inhibit HIV infection in primary macrophages by reducing the activity of luciferase enzyme and increase the expression of Nrf2[55]. In a study conducted by Jazwa *et al.* the activation of Nrf2 by sulforaphane (SFN) in rats with methyl-4-phenyl-1-2-3-6-tetrahydropyridine (MPTP)-induced PD elevates Nrf2 levels in the basal ganglia and a further increase in phase II antioxidant enzymes, HO-1 and NQO1[56]. However, the above results with SFN results were contradicted in clinical studies[41]. Synthetic triterpenoid CDDO-MA activates Nrf2 in wild type mouse embryonic fibroblasts by blocking t-butyl hydroperoxide that induce the production of ROS (fig. 7)[57]. According to a study in RAW264.7 cells, curcumin upon activation of Nrf2-Keap1 pathway, leads to an increase in activity of antioxidant enzymes and showed a bifunctional activity i.e., lowering the ROS production at a lower dose (5μM) and middle-dose (10μM) whereas it intensified the production of ROS at a high-dose (20 μM)[58]. Nrf2 can also be activated via Ceramide-PKCζ-casein kinase 2 signaling pathway that upregulate antioxidant enzymes in the brain[59]. The activation of Nrf2 prevents the transcription of proinflammatory mediators such as TNF-α, interleukin-1 (IL-1), inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2) and intracellular adhesion molecule (ICAM)[60].

The controversial role of NRF2:

Nrf2 is reported to play a crucial role in human carcinogenesis. It has been reported that lung cancer and non-small cell lung cancer (NSCLC) results due to the activation of MEK1/2-ERK1/2-induced apoptosis pathway in endoplasmic reticulum (ER) stress via the activation of unfolded protein response

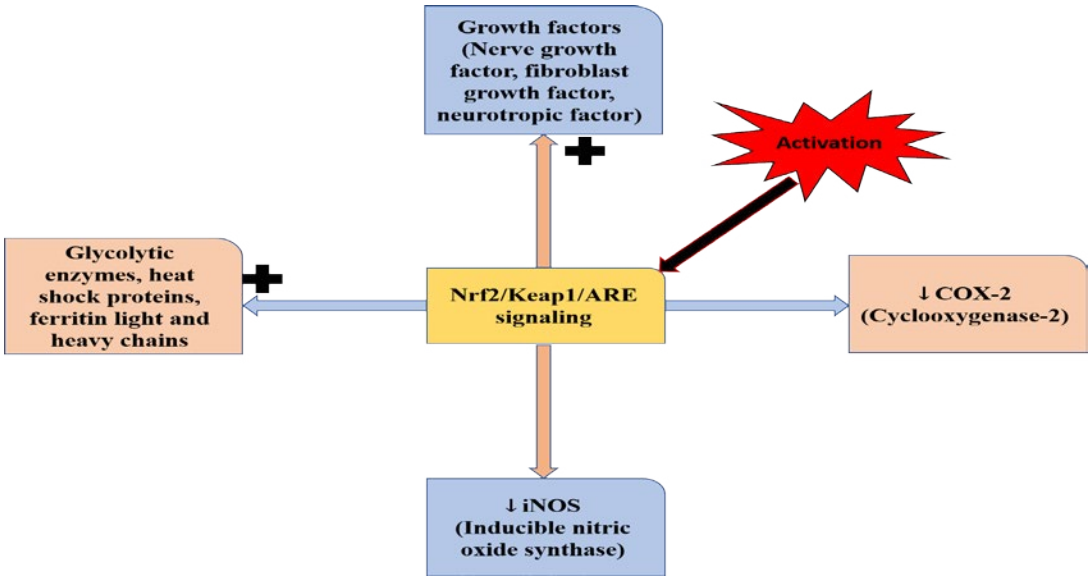

Fig. 7: Activation of Nrf2 by synthetic triterpenoid CDDO-MA

(UPR). Gall bladder cancer is reported to be caused due to the mutations in Keap1 that leads to the nuclear accumulation of Nrf2 leading to a surge in the expression and activity of genes like NQO1 and GST in the case of Keap1-mutated lung tumors[61,62]. In a similar study in NSCLC, by using a combination of erastin and acetaminophen, the regulation of Nrf2/HO-1 signalling pathways has led to ferroptosis due to the overgeneration of lipid peroxidation[63,64]. In a study on head and neck cancer (HNC) using HNC cell lines, it was found out that the overexpression of Nrf2 leads to chemoresistance of HN3 cells to Glutathione peroxidase 4 (GPX4)-inhibitor, RSL3[65]. Similarly, increased nuclear expression of Nrf2 and decreased cytoplasmic expression of Keap1 has been reported in a study conducted among Uighur women associated with cervical squamous cell carcinoma (CSCC) and cervical intraepithelial neoplasia (CIN) due to hypermethylation of CpG islands in the Keap1 gene promoter and further suggested that in cervical cancer tissues epigenetic changes regulate Keap1 expression[66]. These evidences highlights the controversial role of Nrf2 in cancer as it shows protective action in normal and pre-malignant tissues against cancer initiation and progression whereas it enhances the growth of malignant cells [67]. Similarly, in myeloid cells of mice where a high expression of Nrf2 occurs specifically in neutrophils, Nrf2 showed a positive effect in chronic colitis whereas, aggravation of the diseases occurs in acute colitis. These leads to a conclusion that the beneficial effects of Nrf2 activation depends on the organ affected, duration of inflammation, stage of the disease and injury model[68-71].

Conclusion and future perspectives:

ROS are derived mainly from mitochondria. So mitochondrial dysfunctions are often associated with neurodegenerative diseases such as AD, PD, HD and amyotrophic lateral sclerosis. *In vivo* and *in vitro* data have identified that phytochemicals can defend cells against OS-induced cellular damage through activation of Nrf2. This suggests that potential therapeutic targeting of Nrf2 could be beneficial for patients associated with neurodegenerative diseases. The role of Nrf2 in cancer is controversial and therefore thorough studies are required to establish the exact role of Nrf2 in pathogenesis of cancer. Nrf2 activators can play a beneficial therapeutic role in prevention and treatment of diseases associated with OS and in cancer and compounds that inhibit Nrf2 could play a role in chemotherapy of cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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