

Insight into the Regulation of Nrf2/Keap 1 Pathway by Flavonoids as an Approach for Treatment of Liver Diseases: A Review

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ABSTRACT

Around 10% of the world's population suffers from liver disease, making it one of the deadliest diseases in the world. Flavonoids are a class of phenolic substituents present in a wide range of vegetables and fruit and have been identified to control the Nrf2 pathway. Therefore, flavonoids are attracting significance as a therapeutic strategy for liver disease. The main objective of this review discuss the role of flavonoids modulate Nrf2 for the prevention and treatment of liver disease. It has been investigated in relation to several liver illnesses like alcoholic liver disease, cirrhosis, and fibrosis. Flavonoid is among the most widely investigated herbal supplements for preventing and diagnosing liver illness. By triggering the normal ARE target affinity site, Nrf2 encourages the production of metabolic enzymes. It can also enhance the antioxidant and inflammation response and play a significant role in the illness. Oxidative stress play an important role in the pathophysiological changes of liver diseases. Nrf2 is known to participate in hepatic fatty acid metabolism, as a negative regulator of genes that promote liver disease. Nrf2 increases the gene regulation of Heme oxygenase-1 and NQO-1 which initiates an anti-inflammatory response, improves mitochondrial function, and reduces oxidative stress. Nrf2 activated LXR- α activity and LXR- α dependent hepatic steatosis and helps in the regulation of VLDLR in liver disease. This review may stimulate the scientific world to examine flavonoids to develop innovative and effective medications with hepatoprotective effects via altering the Nrf2 signaling pathway.

Keywords: Nrf2, Liver, Flavonoids, Antioxidant, Hepatic disease, Keap1.

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INTRODUCTION

The liver is the biggest organ in the body, which is around 2% of the adult body mass.¹ The liver maintains metabolic processes and detoxifies the body from internal and external threats such as xenobiotics, viral infections, and persistent alcoholism.² One of the most crucial and separate functions of the liver is that it also removes toxins from the blood that are produced by alcohol, chemicals, heavy metals, and organism.³ Hepatic disease is one of the major worldwide health conditions due to wide incidence and poor long-term clinical exposure including early mortality from hepatic disintegration and cirrhosis of the liver and hepatocellular carcinoma.⁴ Hepatic diseases such as viral hepatitis, ALD and

NAFLD with cirrhosis are a significant source of sickness and morbidity worldwide.⁵

Oxidative state and inflammation are the primary pathogenic cause of hepatic disease. Oxidative stress is a condition which involves an imbalance between reactive oxygen species generation and clearance in cells when the cellular antioxidant system is unable to detoxify them.⁶ About oxidative state, cells stimulate the antioxidant pathway to produce the Nrf2, the master controller of antioxidant enzymes.⁷ Phytonutrients obtained from various biological sources have been significant emphasis on drug development, to create unique preventative and therapeutic approaches for a variety of diseases such as cirrhosis, fibrosis, and hepatocellular carcinoma.^{8,9} Flavonoids (flavus-yellow), bioflavonoids, are one of the most significant types of natural ingredients in plants having more than 9,000 demonstrated structures.¹⁰ On the basis of structure arrangement, the flavonoids are categorized into flavone, flavanol, flavanone, isoflavone, and anthocyanins.¹¹ Flavonoids have a lot of healthcare benefits,



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but their poor bioavailability has been a matter of concern.¹² Most flavonoids are assumed to be nontoxic in comparison to a particular secondary metabolite of plants, but their tolerability and pharmaceutical medicament in medical studies are yet not well investigated and determined. Nrf2 is a crucial transcription factor that is strongly connected to inflammation-induced oxidative stress.¹³ Additional research categories revealed the critical regulation of Nrf2 protected the liver from medicine and xenobiotics.¹⁴ Numerous studies identified an interaction between Nrf2 and numerous types of hepatic diseases such as cirrhosis, HCC, ALD, NAFLD, non-alcoholic steatohepatitis, viral hepatitis, and fibrosis.¹⁵ An extensive range of phytochemicals, including flavonoids, have proven the ability to activate the Nrf2/ARE pathway without the presence of oxidative inducers. Flavonoids' desire to protect the liver lead to increased levels of antioxidant defense genes and phase 2 detoxification genes.¹⁴ This review summarises the role of the Nrf2 /Keap1 pathway in hepatic conditions and the effect of natural moiety in hepatoprotection via the enhancement of the Nrf2 /Keap 1 signaling pathway.

Epidemiology of hepatic disease globally and in China

Currently, hepatic disease causes approximately 30,000 to 40,000 patient death each year in China.¹⁶ According to the national statistics in the United Kingdom after heart disease, stroke, chest disease, and cancer, hepatic disease is now the fifth most widely prevalent disease.¹⁷ Around 2 million deaths occur globally from liver disease, of which 1 million are because of cirrhosis, 1 million to viral hepatitis, and 1 million to HCC.^{2,18} The epidemiology of hepatic disease globally and in China is summarized in Table 1.¹⁹ The status of liver diseases in India is mentioned in (Figure 1).²⁰

The canonical Nrf2 pathway

During oxidative stress conditions, cells change their metabolism and gene regulation to preserve redox homeostasis via stimulating NF-E2-related factor 2 (Nrf2) and additional stressors pathways.²¹ An oxidative state occurs because of the collection of reactive oxygen species, such as O₂, H₂O₂, OH, and the second reactive species such as peroxy and R-O*.²² Reactive oxygen species can destroy proteins, lipids, and deoxyribonucleic sequences. Nrf2 is referred to as, a master controller which regulates the transcriptional activation of genes in anti-oxidation, antioxidant pathway, and catabolism pathways.²³

Nrf2

Nrf2 was introduced in 1994 as a regulator of beta-globin regulation.²⁴ Nrf2, belongs to the Cap "n' Collar (CNC) basic leucine-zipper (b-Zip) proteins^{25,26} which control cell response against oxidant and electrophilic state.²⁷ Nrf2 role as a transcriptional influence was introduced in 1996 which helps in the regulation of antioxidants and detoxifies the catalyst and is an activator of ARE.^{28,29} Nrf2 is found in the cytosol.^{30,31} The similarity between NF-E2L2 binding sequences and ARE was first reported by Itoh and colleagues.³² In humans, Nrf2 is made up of 605 AA³³ and contains seven functional domains called Nrf2-ECH homologies (Neh) represented as Neh1-7.³⁴ The Neh domains have a different goal represented in (Figure 2A).³⁵

Neh1 has a basic leucine-zipper (bZIP) structure which plays a key role in deoxyribonucleic acid attachment, as well as Nrf2 dimerization with small muscle aponeurosis fibromatous proteins which play the most important function in interaction with UbCM2 and E2 ubiquitin-conjugating enzyme which is reactive for Nrf2 protein strength.³³ The N-terminal Neh2 domain (AA1-86), is related to cysteine-rich peptides, KELCH-like ECH-associated protein, which is important for the primary ubiquitination E3 ligase adaptor that moderates ubiquitination-dependent proteasomal degradation of Nrf2.³⁶ The Neh2 domain has a high-affinity ETGE and lower-affinity DLG motif to which Keap1 is to be bound.³⁷ The Neh3 domain is essential for the stimulation of the Nrf2 gene through binding with the chromo-ATPase and helicase DNA binding proteins known as CHD6.³⁸ Neh4 and Neh5 provide an interaction site for nuclear cofactor RAC3/AIB1/SRC-3 and CREB-Binding Protein (CBP) and enhance the Nrf2/ARE activation pathway by initiating the acetylation of Nrf2.³⁹ GSK-3 can phosphorylate the serine-rich residue in the Neh6 domain which results in the degradation of Nrf2 by the proteasomal cullin 1 (cul1) dependent ubiquitination.⁴⁰ The most recently described domain is Neh7. It contains a region that can help in protein-protein interaction between Nrf2 and the DNA binding domain of the retinoid X receptor alpha (RXR).⁴¹ This inhibits Nrf2 activity by blocking the recruitment of coactivators to the Neh4 and Neh5.⁴²

Stimulation of Nrf2

Nrf2 interacts with the protein called Keap1 present in the cytosol.⁴³ Keap1 was identified by Masayuki Yamamoto and

Table 1: Epidemiology of hepatic disease globally and in China.

Diseases	Globally (%)	China (%)
Non-alcoholic fatty liver disease	>600 million	200 million (15)
Alcoholic liver diseases	>150 million	60 million (4.5)
Hepatitis B infection	2 million	
Anti-HCV positive	200 million	10 million (<1.0)
HBsAg positive	350-400 million	Million

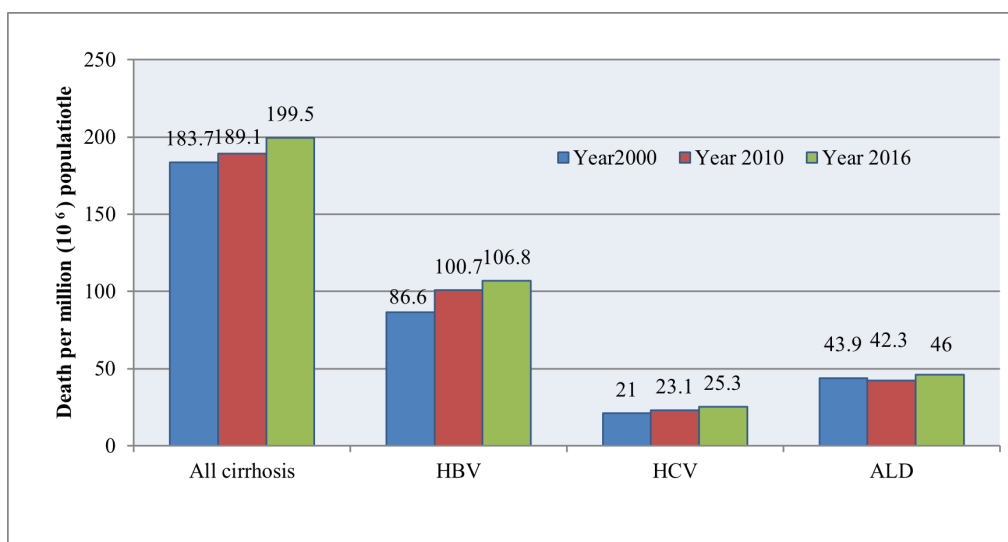


Figure 1: Changes in death rates in all cirrhosis as well as in HBV, HCV, and in ALD.

his colleagues in 1999 as a negative regulator of nuclear factor erythroid 2-related factor 2 and then immediately they turned focused on cysteine residues of Keap1.⁴⁴ Cysteine is different from other amino acids because of its sulfhydryl (thiol) functional group which plays a role in performing different type of functions, which include creating intra-molecular and inter-molecular chemical binding with certain other amino acids, interacting with metallic materials and semimetals and passing from either improvable or irreparable decomposition when it comes into contact with an oxidant.⁴⁵

Keap1 is a 69.7-KD actin-binding protein having 625 amino groups of which, 27 are cysteine residues. Because of cellular redox situations, Keap 1 controls the constant rate of Nrf2 and is known as the master controller of the Keap1 Nrf2/ARE pathway.⁴⁶ Keap 1 is a cofactor, containing three occupational areas: a broad-complex, tram Trafck-Bric-a-brac (BTB homodimerization area), an Intervening Region (IVR), C-terminal KELCH area with Double Glycine Repeat (DGR) area.⁴⁷ In 2014, the arrangement of the Keap 1 Tram track-Bric-a-brac area was reported. They revealed a fold that is identical to the PLZF domain despite also having a low sequence identity and different cellular functions.⁴⁸ The N-terminal Neh2 area of the Nrf2 interacts with Keap1 by both DLG and ETGE motifs.⁴⁹ In the case of the oxidative condition, the DLG motif in Nrf2 released from the DGR domain is Keap1, then inhibiting Nrf2 ubiquitination and subsequent degradation.⁵⁰ According to the Hinge and latch model, KELCH-like ECH-associated protein interacts with the ETGF motif which is longer as compared to the DLG.¹³ The IVR area is crucial for Keap1 cytoplasmic localization just because it carries the binding sequence for fundamental signaling.⁵¹ An intervening region has extremely reactive amino groups which help in ROS conditions, RNS, and H₂S.⁵² The IVR region comprises the most reactive cysteine group of Keap1 (Cys257, Cys272, Cys288,

Cys297) which are the redox sensors.⁵³ The DGR domains have six repeats of the KELCH motif, resulting in the formation of a six-bladed -propeller arrangement. Every blade of the -propeller arrangement is made up of a four-stranded antiparallel beta-sheet, which enables Keap1 for binding with numerous proteins.⁵⁴ The structure of Keap1 is shown in (Figure 2B).⁵⁵

Transcriptional regulation of Nrf2

Nrf2 regulates the transcriptional mechanism when the Nrf2 transcription factor is attached to ARE in the activator area of various tissue against genes to stimulate their transcription. The NFE2L2 gene has an ARE with the activator area for inhibiting the Nrf2 capability so that they immediately stimulate their transcription and carry out a favorable response for increasing the Nrf2 effects.⁵⁶ In addition, it also suggested that Nrf2 regulates various oncogene's signalling pathways such as K-RAS(G12D), B-RAF (V619E) and MYC (ERT2), the PI3K-AKT pathway which further increases the miRNA level of Nrf2 and its target genes.⁵⁷ In the case of the oxidative stress, results in the formation of a binding site for Cullin 3 (CUL3), then introduces Nrf2 into the cullin-ring E3 ubiquitin-ligase complex (CRL) multi-subunit protein arrangement.⁵⁸

Nrf2 Regulation by Gene

More than 500 genes were modulated by the Nrf2/ARE pathway.⁴⁴ The gene target controlled by ARE involves phase 1 and 2 detoxified enzymes, transport proteins, proteasome subunits, chaperones, growth factors, and receptors with transcriptional factors.⁵⁹ Nrf2 target antioxidant enzymes include HO-1, NQO1,⁶⁰ NRH: quinone oxidoreductase 2(NQO)2, Glutamate-cysteine ligase catalytic (GCMC), superoxide dismutase1, glutathione S-transferase (GSH) and so on, which are responsible for the maintenance of oxidant, an antioxidant condition the cells.^{61,62}

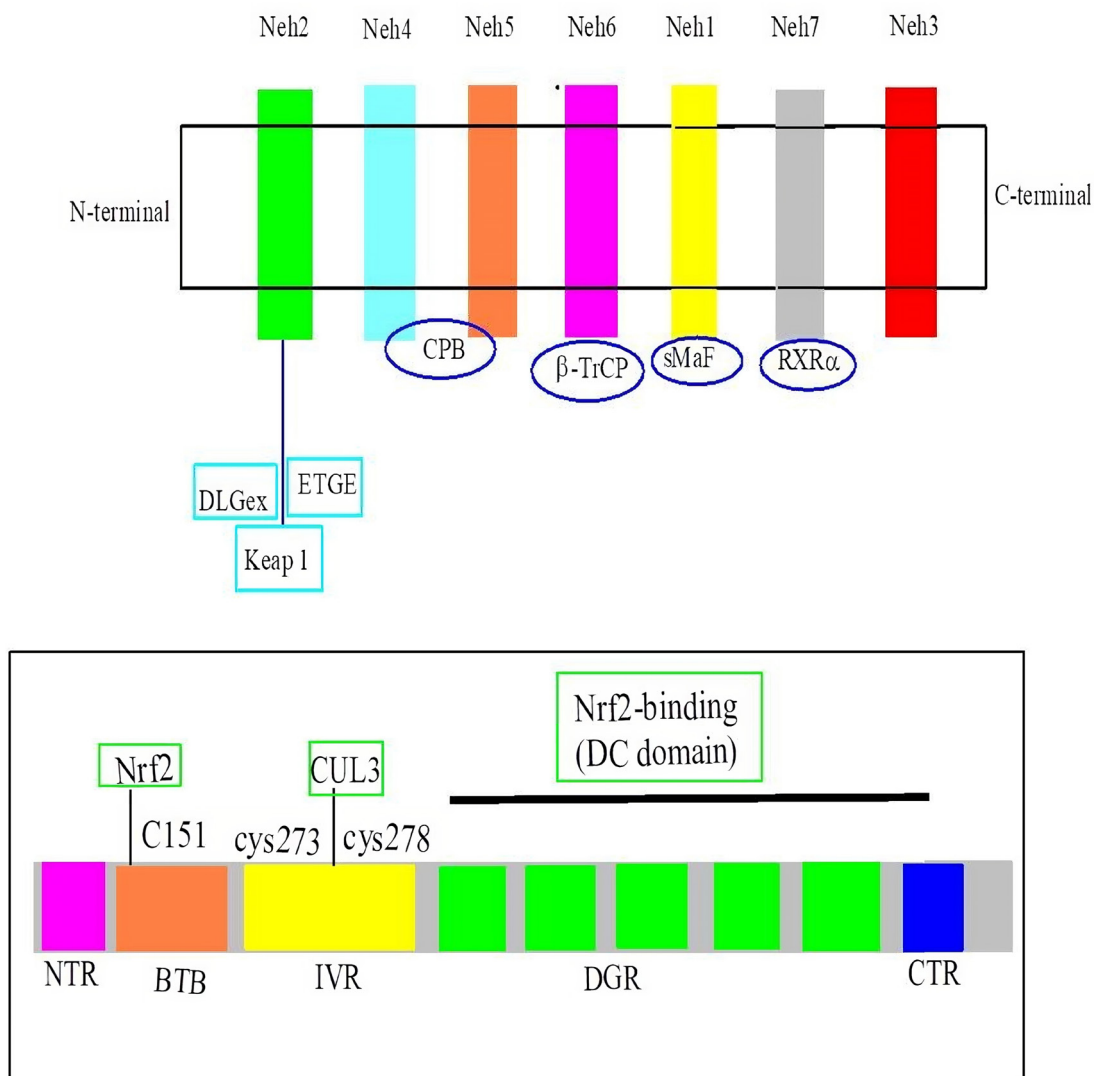


Figure 2: Domain framework of Nrf2, Structure of Keap1.

NADPH-quinone oxidoreductase

NADPH-quinone oxidoreductase is a flavoprotein that competes with cytochrome p450 reductase and detoxifies the enzymes which are responsible for the prevention of cells from quinone-initiate oxidative destruction.⁶³

HO-1

HO-1 is an enzyme and the first step which are responsible for the breakdown of pro-oxidant heme into CO, biliverdin, and ferrous.^{64,65} Heme is a protoporphyrin IX ring having a Fe₂ atom present in the middle which can regulate the Fenton reactor to create extremely dangerous hydroxyl radicals which are derived from hydrogen peroxide.^{66,67} HO-1 has both antioxidants, as well as anti-inflammatory effects when biliverdin is transferred into

the anti-oxidant bilirubin via biliverdin reductase and in a small quantity of CO, may act as anti-inflammatory properties.⁴²

Superoxide dismutase (SOD)

SOD is an enzyme that is responsible for neutralizing the superoxide anion and further, it is considered a biological defense against OS. SOD catalyzes superoxide into H₂O₂ which is catalyzed by glutathione peroxidase enzymatic reaction.² The eukaryotic genome contains three distinct SOD genes: SOD-1 encrypts cytoplasmic Cu/Zn-SOD, SOD-2 encrypts mitochondrial Mn-SOD, and SOD-3 which encrypts extracellular Cu/Zn-SOD. The human SOD-1 and SOD-2, SOD-3 genes are found in genetic code 21q22, genetic code 6q25.3, and genetic code 4. The SOD-2 gene differs significantly from the SOD1 and SOD3 in terms of genetic organisation.⁶⁸

Glutathione-S-transferases (GSTs)

GST superfamily contains various isoenzyme that is present in different location such as in the cytosolic, membrane, and mitochondria. GST is responsible for the oxidative defense and detoxification process because they convert GSH to electrophiles.²⁰

Post-Translational Regulation of Nrf2 and Keap1

The post-translational modification⁶⁹ involves acetylation⁷⁰ and phosphorylation as shown in (Figure 3) which are required for the Nrf2 stimulation. Nrf2 has threonine, serine, and tyrosine residues which enable location for performing phosphorylation by a variety of kinases including⁷¹ mitogen-activated protein kinase⁷² and PKC at Ser 40, Protein kinase-like ER kinase (PERK)⁷³ and Fyn kinase, Phosphatidyl Inositol 3-kinase (PI3K). Nrf2 has a Neh2 domain which can be phosphorylated by protein kinase C at ser-40, which further facilitates the Nrf2 translocation by breaking the binding between Nrf2 and Keap 1.⁷⁴ Phosphorylated Nrf2 is further removed from the cell and then undergoes ubiquitination by -TRCP/cullin 1 E3 ligase complex.⁷⁵ Acetylation of Nrf2 is carried out when p300/CBP⁷⁶ interacts with NRF2 in case of oxidative condition which is further produced via acetylates, arsenite within the Neh1 DNA binding site of Nrf2. This binding site produces no effect on Nrf2 protein stability but affects the capability of Nrf2 with the DNA sequences.⁷⁷ In this way, it affects itself downstream of the Nrf2 /KELCH-like ECH-associated protein complex while increasing the capacity of Nrf 2 for binding with the DNA.⁷⁸

Connection of Nrf2 with liver disease

The liver is a flexible structure that regulates detoxification³ as well as lipid metabolism. Additionally, it also regulates the hepatic antioxidant defensive mechanism, thus Nrf2 changes the energy metabolism pathway in the liver.⁷⁸ Along with hepatic stellate cells, Kupffer cells, and parenchymal hepatocytes, Nrf2 stimulation is also found in non-parenchymal cells.⁷⁹ Alcoholic and NAFLD and HCC, hepatitis B, and hepatitis C are liver illnesses associated with a loss in antioxidant defense.^{80,81}

Role of Nrf2 in Non-alcoholic Steatohepatitis

NAFLD is a continuously developing illness arising from the deposition of lipids in the liver and developed all over the world.⁸² About one-third of individuals suffering from NAFLD in which they develop severe NASH which is related to the inflammatory response⁸³ and fibrosis. NAFLD was connected to obesity⁸⁴ and insulin resistance⁸⁵ which includes NASH.⁸⁶ Oxidative condition is considered the initial cause of hepatocellular injury which promotes liver inflammation and fibrosis in NASH individuals.⁸⁷ Therefore, hydrogen peroxide affects sequences and fatty acid synthesis, causing an inflammatory response, hepatic fibrosis, and apoptosis.⁸⁸ After the deposition of triglyceride in the liver affects the mitochondrial respiratory chain process which causes excess

production of ROS and loss of mitochondrial glutathione. Nrf2 plays a key role in NASH and its activation which protects against NASH disease.⁸⁹ Therefore, Nrf2 activation affects the activation of genes that participate in metabolic order which is crucial for maintaining nutritional homeostasis, particularly during the initiation and progression of NAFLD.⁹⁰ A report established that Nrf2 activation prevents LXR- α activity and LXR- α dependent hepatic steatosis. Liver X receptor- α (LXR) is a member of the nuclear receptor family of ligand-dependent transcription factors.⁹¹ It promotes the synthesis of new fatty acid formation in steatohepatitis patients.⁹² The main immune cells include Kupffer cells, dendritic cells, neutrophils, and natural killer cells that help to encourage non-alcoholic fatty liver disease/NASH initiation via detecting DAMPs secreted from destroyed hepatocytes or PAMPs produced from many other tissues including the gut and adipose tissue.⁹³

According to the previous study, activation of Nrf2 increases the gene regulation of Heme oxygenase-1 and NQO-1 which further initiates anti-inflammatory effects. HO-1 is demonstrated in mice to suppress the phosphorylation of nuclear factor- κ B by its own substrate tumor necrosis factor revealing that it has inhibitory effects on the beginning of the inflammatory activity.⁶⁵ Another pathway for a potential NAFLD therapy is the activation of Nrf2, which help in the regulation of transforming growth factor- β .⁹⁴

Green tea extract and ezetimibe are identified with hepatoprotective action on Nrf2 in contrast to fat accumulation and soreness in NASH by p62-dependent modulation of Nrf2. The p62 accumulation causes Nrf2 to stabilize and accumulate while disrupting the Keap1 and Nrf2 interaction.⁶ Scutellarin (*Erigeron breviscapus*) is a herbal medication with a constituent of breviscapine, which helps in increasing the Nrf2-initiated by reducing the hemoglobin fat level and increasing antioxidant level by stimulating the PPAR γ and its coactivator-1 α , Heme oxygenase-1, glutathione-S transferase, NQO-1 and thus decreasing the nuclear factor- κ B and KELCH-like ECH-associated protein at the protein level thus improving the non-alcoholic fatty liver disease.⁹⁵ Punicalagin is a vitamin used in the prevention of non-alcoholic fatty liver disease because it activates Nrf2, which improves mitochondrial function and reduces inflammation and oxidative stress.⁹⁶

Role of Nrf2 in Alcoholic Steatohepatitis

The alcoholic liver disease involves a variety of liver diseases, from moderate metabolic abnormalities (steatosis) to serious conformation of hepatic diseases like alcoholic steatohepatitis, HCC, and cirrhosis.⁹⁷ A major factor that helps in the progression of alcoholic-induced hepatic illness is the chronic consumption of alcohol, which increase the formation of reactive oxidants.^{98,99} It is metabolised by three enzymatic pathways:¹⁰⁰ alcohol dehydrogenase oxidises ethanol in hepatocytes, and cytochrome P450 2E1(CYP2E1) catalyses microsomal oxidation.⁹⁷ Fatty acid

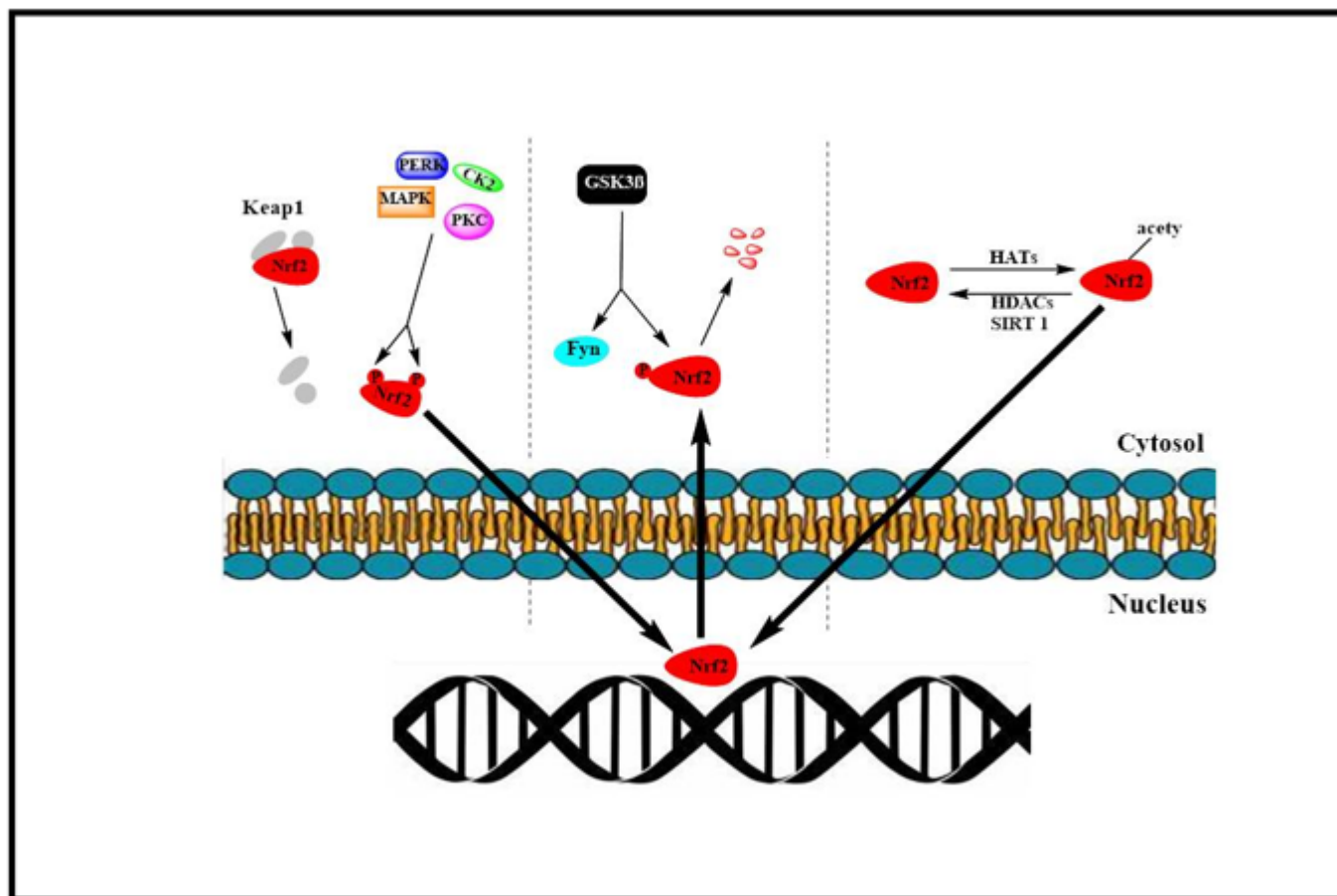


Figure 3: Post-translational of Nrf2.

ethylene synthase catalyzes non-oxidative metabolism. Alcohol is broken down by the enzyme CYP2E1 into ROS such as H_2O_2 and O_2 . Additionally, by raising the NADH/NAD ratio, alcohol metabolism causes fatty liver disease. When CYP2E1 (E47 cells) is over-expressed in Hep G2 cells, Nrf2 miRNA and protein levels are higher than in control Hep G2 cells (C34 cells). The nuclear translocation of Nrf2 and Nrf2-ARE binding affinity is enhanced in E47 cells and Nrf2 helps in the regulation of genes like GCLC and HO-1.¹⁰¹

Nrf2 stimulation is further effective in opposition to ethanol-induced hepatic fibrosis and liver toxicity.²¹ Nrf2 activation by 1,2-Dithiole-3-thione (D3T) is used to investigate the impact of Nrf2-induced antioxidant factors in mice exposed to ethanol.^{102,16} D3T reduced the production of ethanol-induced reactive oxygen species and apoptosis, which shows that initiation of Nrf2 can destroy ethanol-initiated apoptosis.⁶ By initiating the Nrf2-mediated signaling pathway, the coupling of curcumin and baicalin is used for the prevention of ALD by enhancing the downstream antioxidant enzyme NQO-1 and HO-1 expression.¹⁰³ Sulforaphane is an activator of Nrf2 which is found in brassica vegetables involving broccoli, and cabbage, and is further helpful in preventing alcohol-initiated liver steatosis.⁶ Lastly, Nrf2 signaling effectively enhances the regulation of the

Very Low-Density Lipoproteins Receptor (VLDLR), which helps in the progression of liquor initiates damaging to the hepatic.¹⁰⁴

Viral hepatitis

Viral hepatitis represents one of the most widespread liver diseases worldwide.⁵ Global Health Sector Strategy (GHSS) on viral hepatitis 2016-2021 was approved by the World Health Assembly in May 2016. According to GHSS, viral hepatitis may not be dangerous to the population by 2030, with decreasing contamination by 90% and mortality by 65%.¹⁰⁵ Viral hepatitis having types A, B, C, D, G, and E, types B and C most commonly cause liver cirrhosis and fibrosis. Hepatitis B Virus (HBV) and Hepatitis C virus (HCV) infection are the indicator factors in the development of persistent liver disease.⁷⁴

Nrf2 in Hepatitis B (HBV)

HBV is the smallest DNA, belonging to the family of *Hepadnaviridae* and the genus *Orthohepadnavirus*.¹⁰⁶ Hepatitis B has four genomes: the polymerase, surface proteins (LHBs, MHBs, SHBs), and the core protein containing the regulatory protein HBx its secretory variant HBeAg. Chronic Hepatitis B can initiate hepatic fibrosis and cirrhosis, and it is also responsible for HCC.¹⁰⁷ HBx is a factor that helps in initiating the production of

ROS. Hepatitis B includes HBx and LHBs, both are responsible for activating the NF- κ B which results in the formation of pro-inflammatory cytokines.¹⁰⁸ Additionally, HBx also interacts with p62 which traps Keap1¹⁰⁹ and prevents it from interacting with Nrf2, thus stabilizing Nrf2, and enabling its translocation to the cell nucleus.¹¹⁰ Thus, Nrf2, is stimulated because of HBx p62 Keap 1 complex formation as shown in (Figure 4).¹¹¹ The initial steps in the activation of c-Raf are triggered by both regulatory proteins (HBx and LHBs).¹⁰⁸ The HBV-dependent stimulation of Nrf2, was associated with the cRaf stimulation.¹¹² It was also demonstrated that when the human HBV genome was inserted into the stable cell lines HepAD 38 and HepG2.215, the regulation of numerous Nrf2-ARE pathway-regulated cytoprotective genes was elevated both *in vitro* and *in vivo* in comparison to HBV-negative HepG2 cells.¹¹³

Role of Nrf2 in Hepatitis C Virus (HCV)

Hepatitis C virus is a positive-strand RNA virus belonging to the *Flavivirus* family within the genus *Hepacivirus*.^{114,115} Hepatitis C infections are the primary initiation of chronic liver disease globally involving cirrhosis, fibrosis, and HCC.¹¹⁶ In human liver resident cells, HCV is linked to oxidative stress, chronic HCV patients have considerably higher levels of oxidative state markers such as malondialdehyde, nitric oxide, and myeloperoxidase

activity. HCV causes a larger level of ROS production as compared to certain viral pathogens; individuals who suffer from permanent hepatitis C have more than an 80% chance of increased acquiring the chronic illness.¹¹⁷ Human HCC cell line, HCV infection of Huh-7 cells which increased reactive oxygen species generation, and nuclear translocation of Nrf2 in a time-dependent manner, causes the production of ARE-regulated genes, which protect the cell protein from oxidative stress.¹¹⁸ The phosphorylation and resulting nuclear translocation of Nrf2 in HCV-infected cells are initiated by a mitogen-activated protein kinase, casein kinase 2, phosphoinositide-3 kinase, and PKC.¹¹⁹

According to the report, curcumin suppresses the PI3K/Akt-SREBP-1c pathway, which prevents the hepatitis C virus from replication. Additionally, it also found that curcumin can reduce the chances of hepatitis C virus-related hepatocellular by its ability to defend against HCV infection.¹²⁰

Role of Nrf2 in hepatocellular carcinoma (HCC)

HCC would be the fifth-highest widespread cancer in the world, 800,000 cases identified each year.¹⁰⁶ The malignant transformation of hepatocytes is usually induced during chronic inflammation and followed by liver fibrosis resulting in the formation of HCC.¹²¹ Nrf2 also helped in the conservation of

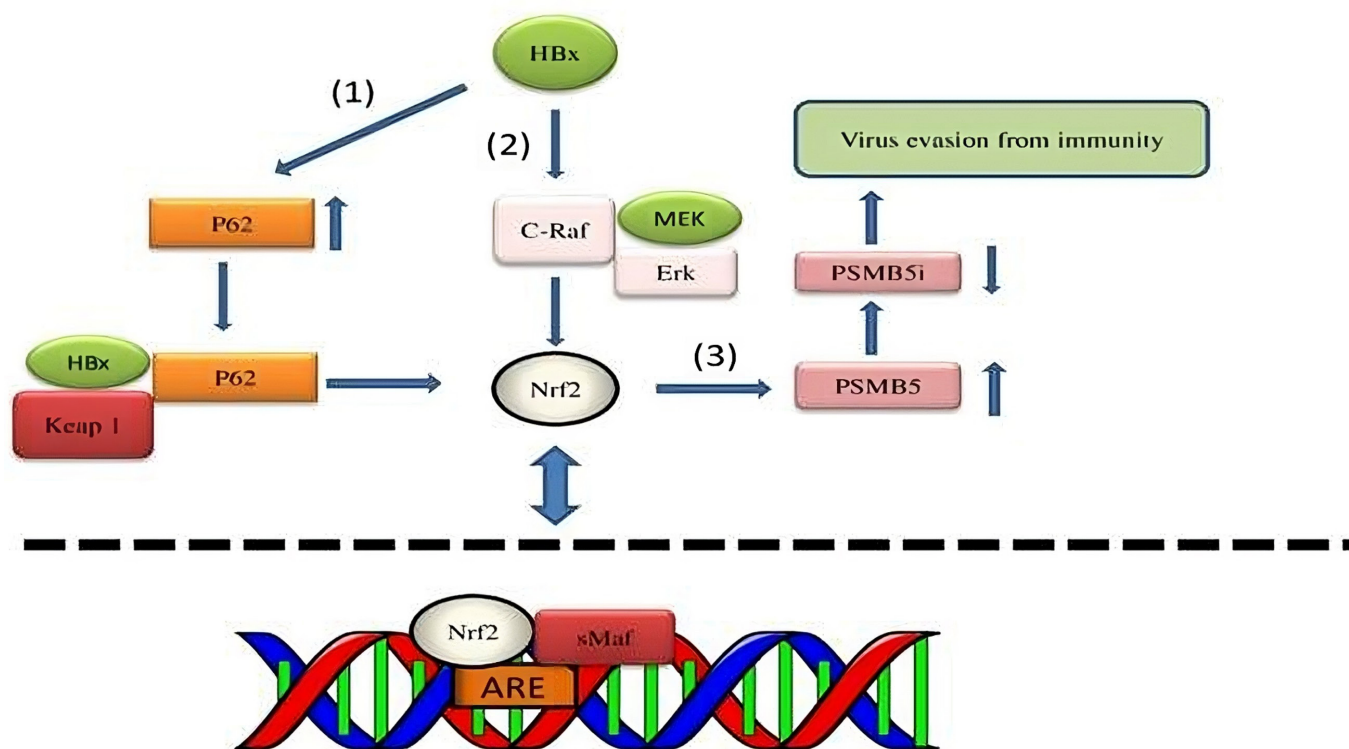


Figure 4: Involvement of Nrf2 in HBV stimulation. (1) HBx protein promotes viral replication, the growth of HBV correlated with hepatocellular carcinoma, the viability of the contaminated cell, and the demonstration of the contamination by activating the c-Raf-MEK-Erk signal transduction pathway and then activating Nrf2. (2) HBx increases p62 and then interacts with Keap1 resulting in the formation of HBx-p62-Keap1 complex in the cytoplasm. (3) Stimulation of Nrf2 by enhancing the regulation of lysosomal component PSMB5 in HBV-positive tissues and decreasing immunoproteasomes causes antigen activity and removal from the immune response.

Table 2: Flavonoids have antioxidant activity.

Compound	Core structure
Morin	R ₃ , R ₅ , R ₇ , R ₄ , R ₆ =OH
Dihydromyricetin	R ₃ , R ₅ , R ₇ , R ₃ , R ₄ , R ₅ =OH
Quercetin	R ₃ , R ₅ , R ₇ , R ₈ , R ₄ =OH
Luteolin	R ₅ , R ₇ , R ₃ , R ₄ =OH
Baicalin	R ₅ , R ₆ , R ₇ =OH
Chrysin	R ₅ , R ₇ =OH
Apigenin	R ₅ , R ₇ , R ₄ =OH

hepatocellular carcinoma cells by enhancing the reaction of FGF1g to endoplasmic reticulum condition.¹²² The oxidative state promotes TGF- which is correlated with the intensity of cell injury and hepatic fibrosis.¹²³ TGF- increases ROS levels by preventing the formation of GSH and antioxidant enzymes, as well as NOX4 which is NADPH oxidase.¹⁵ TGF- β plays an important part in the development of HCC by inducing apoptotic, antiapoptotic cellular pathways.

Capsaicin (trans-8-methyl-N-vanillyl-6-zonisamide) would be a homovanillic compound that is present in red-hot peppers that possess chemopreventive effects.¹²⁴ When Hep G2 cells are treated with capsaicin, then it helps in the production of ROS, further, they encountered NQO-1 decreased its enzyme activity. As a result, increased reactive oxygen species initiate the PI3K-Akt signaling pathway via the phosphorylation of Akt, which helps in the stimulation of Nrf2. Thus, capsaicin inhibited the NQO-1 activity, proceeding with the formation of ROS in HepG2 cells.¹²⁵ Tussilagonone is a compound derived from the medicinal plant *Tussilago farfara L* could activate further and move Nrf2 into the nucleus. NQO-1, a phase 2 detoxifying enzyme, was activated by TGH in Hepa1c1c7 and BPrcl cell lines. Thus, tigloylgomsin can be used for the prevention of HCC.¹²⁶

Role of Nrf2 in hepatic cirrhosis

Hepatic cirrhosis is a pathologic disease in which normal liver cells are removed by granulation tissue.^{127,128} Cirrhosis is defined as a deposition of ECM like gelatin1,3,4 and Alpha-Smooth Muscle Actin (α -SMA) which eventually leads to cirrhosis of the liver. Most commonly it is caused by the accumulation of alcohol consumption, viral hepatitis, long-term use, and exposure to hepatic chemicals.¹²⁹ It was demonstrated that alcoholic cirrhosis helps in increasing Nrf2 miRNA expression as compared to the normal liver.¹³⁰ Recent research revealed that miRNA200a targets Keap1, along with miRNA dysregulation in liver fibrosis and these results help in the degradation of mRNA Keap1 in the activated hepatic stellate cells.¹³¹

Role of Nrf2 in hepatic Fibrosis

Hepatic fibrosis is characterized by an excessive amount of deposition of inflammatory scar in the hepatocellular throughout

the healing process which further result in the formation of cirrhosis and liver failure and sometimes also cause carcinoma.¹³² Hepatic fibrosis occurs because of the imbalance between the breakdown and production of extracellular proteins which further causes the deposition of ECM¹³³ whereas the stimulation of HSCs.¹³⁴⁻¹³⁶ Hepatic stellate cell is pericytes that are present in the perisinusoidal region and play a role in hepatic fibrosis, which leads to the development of granulation tissues with hepatic damage.¹³⁷ Nrf2 stimulation may prevent reactive oxygen species which activate the NLRP3 inflammasome and decrease the IL-1 and TGF-, which suppress the HSC stimulation and accelerate the collagen degradation. Additionally, the antioxidant enzymes and Nrf2 /ARE activate the regulation of mitochondrial-dependent apoptotic proteins such as B-cell lymphoma-2(Bcl-2), Bcl-2 associated X protein (Bax), and caspase-3 which help in reducing the mitochondrial dysfunction, liver cell injury, fibrosis.¹³⁸ It was found that morin may protect by increasing the activation of Nrf2 and its upstream anti-oxidant factors such as HO-1 and NQO-1 by decreasing the production of α -SMA, collagen I, and collagen III in CCl₄-induced liver injury.¹³⁹

Structure-Activity Relationship of Flavonoids as an Antioxidant

Flavonoid

Flavonoids are 1,4-benzopyrone compounds having pyrene and phenolic rings. The chemical nature of flavonoids is characterized by the degree of hydroxylation and degree of polymerization and another substitution and conjugation.¹⁴⁰ A phenolic hydroxyl group is present in the ring, flavonoids containing antioxidant properties as free radical scavengers and hydrogen donating properties, and singlet O₂ scavengers and alloy chelates.^{141,142} The activity of flavonoids as an antioxidant is changed via the no and distribution of the hydroxyl group and the A and B rings as well as the degree of conjugation in the middle of the C and B rings. The glycosylation reduces the antioxidant properties of phytochemicals. Flavonoids possess antioxidant effects because of the immediate scavenging of ROS and accomplishment as ions scavenging properties¹⁴³ because of sharing of a hydrogen atom. These antioxidants further act as a single-electron transfer.¹⁴⁴ By reacting with the reactive elements of the ions, flavonoids stabilize

the ROS radicals that are inactive due to the strong affinity of the OH of the phytonutrients.¹⁴⁵ The common structure of flavonoids is shown in (Figure 5A).

These are some of the flavonoids which show antioxidant activity as demonstrated in Table 2.¹⁴⁶

Structural Characteristics of Flavonoids

Flavonoids that show antioxidative activity have some of the following structural characteristics as shown in (Figure 5B).

The o-trihydroxy (3',4',5') thiol compound present in the structure phenyl ring is responsible for the greatest affinity of the phytonutrients for the phenoxy radical by hydrogen bonding.¹⁴⁷

The OH is located at both 3 and 5 and is important for the maximal radical scavenging activity.¹⁴⁸

Due to the presence of a double bond at C2-C3 double bond which is beneficial for antioxidant properties and anti-viral activity.¹⁴⁹

Functional Group present in Flavonoid

Hydroxyl Group

The Hydroxyl group is present in the B-ring which is responsible for donating a hydrogen atom to peroxy and hydroxyl and peroxy nitrite, which stabilizes and produces stable phytonutrient radicals.¹⁵⁰ In accordance with the overall no of hydroxyl groups, the arrangement of flavones and flavanones will increase continuously.¹⁵¹ B ring contains 3',4', and 5', catechol moieties in the structure which are responsible for lipid peroxidation. In the case of A ring, its substitution pattern shows an antioxidant effect. Presence of OH at 5,7 shows antioxidant activities. A ring containing 3-OH is responsible for various activities such as antioxidant and anti-radical activity.¹⁵²

C2-C3 double bond and 3-Hydroxyl group and 4-Keto group

When it comes to flavonoids containing a thiol moiety on the B-ring, in the C-ring loss of any functional group, the 4-keto group, the C2-C3 double bond, 3-hydroxyl group moiety proceeds to decrease antioxidant activity.¹⁵³ When A and B rings are conjugated then it allows the aromatic ring may exhibit a resonance effect that stabilizes the flavonoid radical therefore it is crucial for carrying out the 3',4'-catechol moiety to stabilise phenoxy radicals.¹⁴⁰

Glycosylation

Glycosylation at C6 and C8 positions may increase the antioxidant activities by enhancing solubility, whereas Glycosylation at O3 and O7 can improve certain activities by increasing the solubility of flavonoids.¹⁵⁴

Effect on intracellular antioxidant enzymes

The mechanism of Flavonoids is dependent on the intracellular activities of enzymes similar to Superoxide dismutase, CAT, and GSH-Px.¹⁵⁵

Flavonoids and Nrf2 Modulation

Flavonoids are most abundantly found in various types of fruitages, comestibles and ingredients of vegetable having nutritional products and significant roles in the prevention of a wide range of disease¹³⁹ like anti-bacterial, anti-viral, anti-inflammatory, anti-ulcer, anti-stress, anti-atherosclerosis, anti-tumour properties apart from these activities, flavonoids are commonly considered as the most effective natural antioxidant found in plants. The diversity of the flavonoid structure was influenced by the replacement of different areas and no of OH, CH₃O and glycoside on the fundamental carbon backbone of C6-C3-C6.¹⁵⁶

Studies have indicated that flavonoids can stimulate Nrf2¹⁵⁷ and ARE in different ways.^{158,159} Nrf2 nuclear translocation to the nucleus and activation of Nrf2 to the ARE both are facilitated by flavonoids. Li *et al.*, in 2018 used the NAD(P)H Quinone Reductase (QR) assay for examining the possible Nrf2 stimulating effect of different flavonoids categorised as flavones, flavonoids, dihydro flavanols, isoflavones, dihydrochalcones in marine hepatoma Keap1 C7C cell. Flavonoids especially flavanol, have a variety of health benefits including the ability to protect the liver from ALD, NAFLD, others.^{160,161,162} Flavonoids also inhibit the activities of control molecules that initiate the inflammatory process, including phosphodiesterase, phospholipase A2, lipoxygenase and cyclooxygenase (COX).¹⁶³ Flavonoids target several signalling pathways including protein kinase B (Akt/PKB), phosphatidylinositol-3 kinase and mitogen-activated protein kinase.¹⁶⁴ The Flavonoids and Nrf2 modulation are shown in Table 3.

Quercetin

Quercetin belongs to the flavanol subclass, which contains oxygen (a carboxyl group) at the 4-position of the heteroaromatic carbon chain and contains double bonds present at 2 and 3 positions.¹⁷⁷ It also contains five classes of OH groups 3,5,7,3',4'.¹⁷⁸ Quercetin is also known as an antioxidant and reactive oxygen species which helps in reducing alcoholic cytotoxicity in the liver which is induced by Nrf2. Quercetin has been demonstrated to promote the phase 2 detoxification of enzymes such as HO-1, NQO1, and Glutathione S-Transferase (GST).¹⁷⁹ According to reports, quercetin enhanced the regulation of Heme oxygenase-1 by inducing ARE to attach with the Nrf2 in the Heme oxygenase-1 gene promoter region.¹ Further, it also enhanced Heme oxygenase-1 activity in LPS-treated rats, D-galactosamine by suppressing haemoglobin concentration of alanine aminotransferase, affecting the liver cytotoxicity and liver

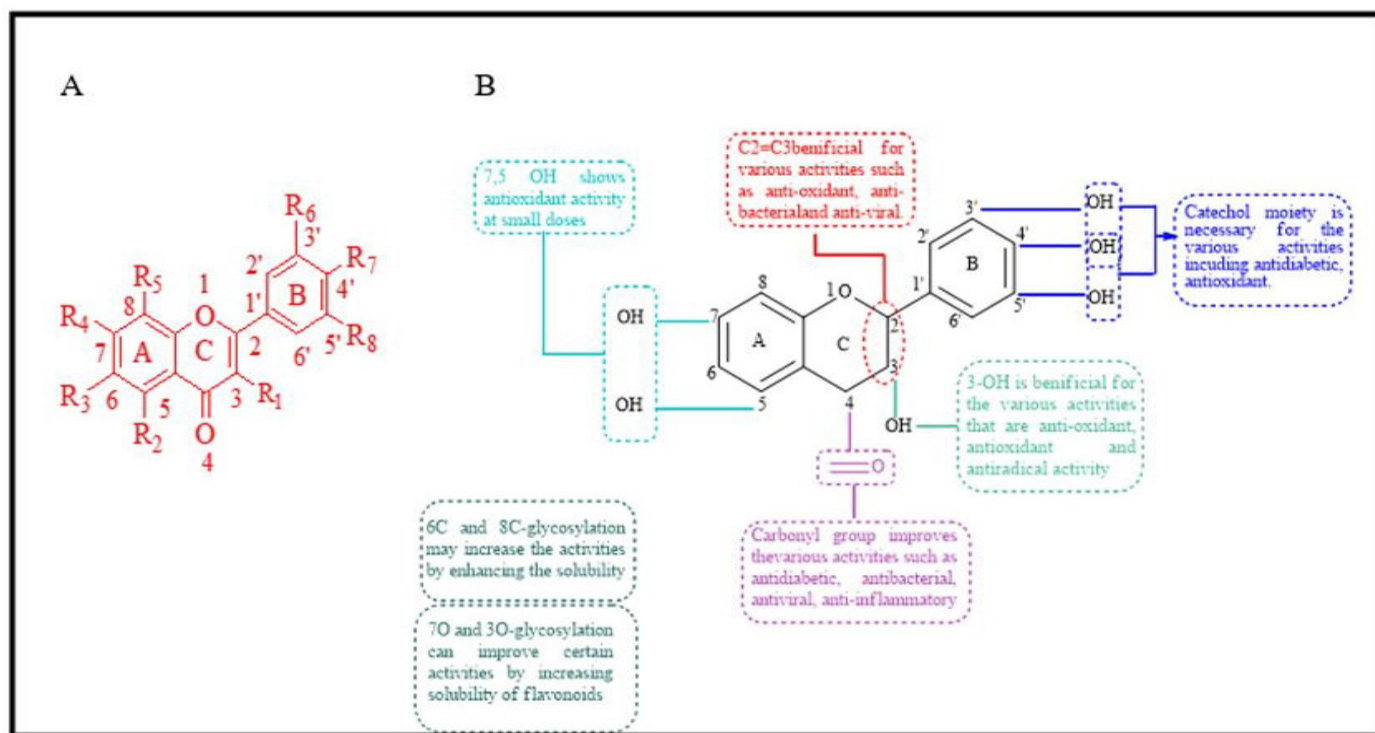


Figure 5: General structure and SAR of flavonoid.

protection activity. Finding the capability of quercetin to repair the alcoholic-initiated peroxidation in rat hepatocytes, indicates that it may be suitable as a hepatoprotective natural product.¹⁸⁰ New research studies discovered that quercetin combines with Keap1 and prevents it from interacting with Nrf2.¹⁸¹ In many plants, quercetin reduced the hepatotoxicity caused by CCl_4 primarily by increasing the regulation of detoxifying the enzymes in the mouse liver. Quercetin also increases the transcriptional level of Nrf2 and Thioredoxin (Trxs), and Peroxiredoxins (Prxs). According to *in vitro* research on the L-02 cell lines found that quercetin may protect the liver against hepatotoxicity initiated by various agents such as aminophenol, and clivorine by the regulation of Nrf2 and JNK.¹⁸² HO-1 activation, particularly by the ERK/Nrf2 transduction mechanism further influences the action of quercetin via p38.¹⁸³

Baicalin

Baicalin is a phytonutrient found in a Chinese medicinal plant known as a *Baikal skullcap* or *Chinese skullcap*.¹⁸⁴ Chemically, it is known as 5,6-dihydroxy-2-phenyl-4H-1-Benzopyran-4-one-7-O-D-B-glucuronic acid¹⁸⁵ it contains anti-oxidation and anti-inflammatory pharmacological activities.¹⁸⁶ Along with the cytochrome p450, Baicalin potentially modifies the transcription factors such as NF-B and Nrf2, which are significant mediators of inflammatory and anti-oxidant defensive systems in alcoholic-initiated hepatic damage *in vitro*, baicalin also regulates the stimulated Keratinocytes cells.¹⁸⁷ Baicalin regulates the HO-1 expression to resist ethanol-initiated hepatic destruction and apoptosis as well as necrosis of hepatocytes.¹⁰³ In

acetaminophen-initiates L-02 human liver cells, baicalin regulates the Nrf2/ARE signalling pathway via non-canonical stimulation of Nrf2 via p62.¹⁶⁰

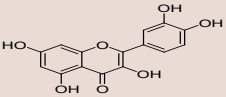
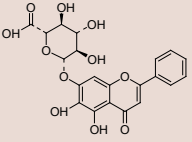
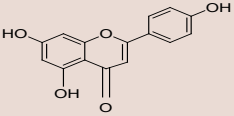
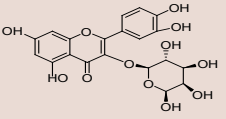
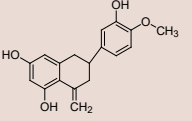
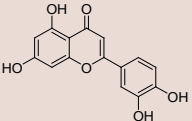
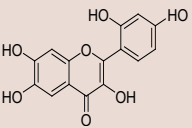
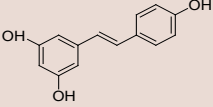
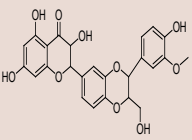
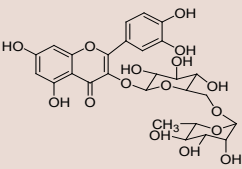
Apigenin

Apigenin (4,5,7, tri-hydroxy flavone) is extracted from fruits and leafy vegetables, enhancing antioxidant properties and protecting against HFD-initiated liver steatosis in rats, initiating the protective effects of hepatic steatosis.^{188,189} Apigenin reduced the HFD-induced effects which are an increase in TG, attenuated Nrf2 translocation into the nucleus, enhanced regulation of antioxidant proteins such as SOD, peroxidase and GSH-Px and changed pathological response (steatosis, lipid droplets in rats). Apigenin prevents the hepatic organ from ethanol-initiated damage, and HFD-induced hepatic toxicity through the activation of Nrf2.¹⁹⁰

Hyperoside

Hyperoside is extracted from *Drosera rotundifolia* L., seeds of *Hypericum perforatum* L., and *Cuscuta Chinensis* Lam generally, having many bioactive characteristics like as anti-thrombotic, anti-viral and anti-inflammation, anti-fungal, hepato-protective, and especially it possesses anti-oxidative properties.⁸¹ Hyperoside also called quercetin O-glycoside and is a vivid yellow, its aglycone is quercetin. Hyp belongs to the genus *Crataegus*, and it is closely related to the *Hypericaceae*, *Rubiaceae*, and *Lamiaceae*.¹⁹¹ Hyperoside acts as an adequate hepato-protective agent by lowering hepatic damage because of oxidative stress which was initiated by chemicals such as carbon tetrachloride

Table 3: Flavonoids and Nrf2 modulation.

Compound	Structure	Sources	MOA	References
Quercetin (Flavanols)		Onion, curly leeks, Broccoli apples, tea, capes, blueberries with onion, <i>R. arboreum</i> .	Increase SOD and up-regulating HO-1.	165,166
Baicalin (Flavones)		Scutellarin braicalensis Georgi.	Nrf2-initiated antioxidant activity for protecting hepatocytes from inflammation, fibrosis.	167
Apigenin (Flavone)		Parsley, chamomile, celery, vine-spinach, and leaves of <i>A. fistulosum</i> .	Nrf2-PPAR γ decrease, increase mRNA expression of Nrf2.	168
Hyperoside (Flavonol)		<i>Saccharomyces cerevisiae</i> leaves.	Regulating the expression of nrf2 in liver fibrosis.	169
Hesperidin (Flavanone)		Sweet orange, lemon Citrus unshiu, Citrus mitis, Citrus sinensis.	Increased mRNA expression and increased Nrf2 and HO-1.	170,171
Luteolin (Flavone)		Celery, Parsley, chrysanthemum flowers.	Increase nuclear translocation of Nrf2.	172
Morin (Flavanols)		<i>Morus aliba</i> (white mulberry), <i>Psidium guava</i> L. (guava leaves).	Hepatoprotective activity via activating Nrf2/HO-1.	173
Resveratrol (Flavanols)		Peanuts, Grapes, Red wines (<i>Polygonum cuspidatum</i>).	Increase the nuclear translocation of Nrf2 and help in HCC pathogenesis.	174
Silymarin (Flavanones)		<i>Mariaum Silybum</i> (Milk thistle).	Activated in HSC by increasing the translocation of Nrf2.	162
Rutin (Flavonol)		<i>Ficus</i> species.	Increase of Nrf2, HO-1 and AMPK activity.	175,176

CCl₄, H₂O₂ and tert-butyl-hydroperoxide (t-BHP). Hyperoside lowers the H₂O₂-induced liver cell injury via the modulation of the Nrf2-ARE, enhanced GSK-3 β phosphorylation at Ser-9.⁸¹

Hesperidin

Hesperidin, also called 3',5'-hydroxy-4'-methoxy-7-rutinosyloxyflavan-4-one, a bioflavonoid and flavanone glycoside which is made up of the 2-phenyl-4-chromone hesperidin attached

to the cellulose 6-O-(6-deoxy--L-mannopyransosyl)-D-glucose. It is a nutrimental substance found in oranges, tangelos, and tangerines.¹⁹² Hesperidin also called hesperidoside, has B ring attached to the methoxy group and hydroxyl substituents and contains phenolic OH groups, which help iron for decreasing the generation of ROS and RNS.¹⁹³ It possesses various pharmacological properties including antioxidant, anti-inflammation, hepatoprotective as well as anti-tumour

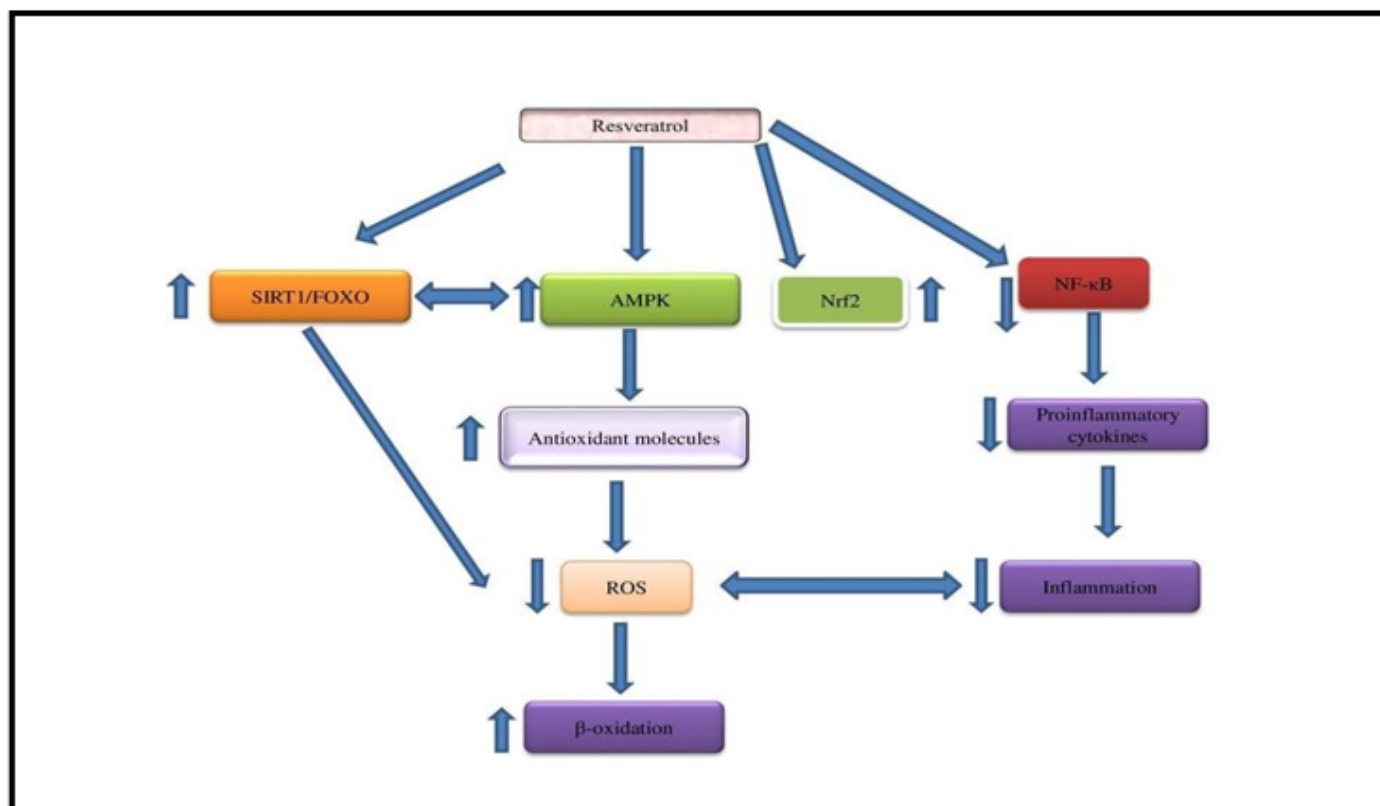


Figure 6: Demonstrated the mechanism by which resveratrol initiates Nrf2 transcriptional activity.

properties.¹⁹⁴ Hesperidin modulates the Heme oxygenase-1 via the stimulation Nrf2/MAPK/ERK pathway.¹⁹⁵ Hesperidin prevented CYP-initiates inflammation and lipid level via increasing hepatic PPAR expression and decreasing NF- κ B expression. PPAR also, help in the protection in opposition to the inflammatory and oxidative state by the stimulation of the Nrf2 pathway and nuclear factor kappa-B stimulation.¹⁹⁶

Resveratrol

Resveratrol is also known as a 3,4,5-stilbenetriol combination¹⁹⁷ and present in shrubs and fruits like grapes and is also established in the root of Indian poke, Japanese knotweed¹⁹⁸ which is well-known for its antioxidant properties and betatrophin. Resveratrol also prevents Liver X Receptor alpha (LXR)-dependent liver adipogenicity by anti-oxidant properties.¹⁹⁹ Resveratrol activates Nrf2 via transcriptional activity by protecting the nucleus from oxidative stain both *in vivo* and *in vitro* depending on the Nrf2.²⁰⁰ Resveratrol enhanced the interaction between Nrf2 and p62 thus separating the interaction between Nrf2- KELCH-like ECH-associated protein. It also stimulates Nrf2/ARE pathway by initiating the p38 MAPK and SIRT1/FOXO1 pathway.¹⁵⁸ Resveratrol increased the enzymatic activities of antioxidant enzymes like NQO-1, GPx, GST, and SOD in hepatocytes.²⁰¹ Resveratrol enhanced Nrf2 transactivation and stimulated the regulation of HO-1 and paraoxonase-1 in liver

(HUH7). The mechanism of Resveratrol is illustrated in (Figure 6).²⁰²

Morin

Morin is a flavonoid which play an important role in many activities such as antioxidants, hepatoprotection, and decrease blood sugar. Chemically it is known as (2-(2,4-dihydroxy phenyl)-3,5,7, -tri-Hydroxy chrome-4-one). It is extracted from various fruits such as *Macluria porifera* (Osage orange), *Maclura tinctoria* (old fustic) and the branches of *Psidium guajava* (guava). Morin protected hepatocytes from the toxicity of acetaminophen and the response was connected to the reactivation of Nrf2 in culture cells. The inhibition of Nrf2 ubiquitination as well as an enhancement in nuclear Nrf2-retention and interaction with the capacity of ARE/Nrf2.²⁰³ Additionally, it improves cellular defence by decreasing the oxidative condition, which inhibits the activation of GSK-3 and Fyn kinase.²⁰⁴

Silymarin

Silymarin is isolated from the milk thistle plant,²⁰⁵ Mary's thistle (*Silybum marianum*).⁸⁴ Silymarin contains the major constituents of six flavonolignans such as (Silybins A and B, Isosilybins A and B, silychristin and silydianin).²⁰⁶ Silymarin is also called an antioxidant that might promote Nrf2 translocation when the MCD diet is administered to a mouse liver and further used in

hepatic treatment. Silymarin is recognized under the phase 4 clinical trial for the treatment of NAFLD and NASH.⁸⁰

Rutin

Rutin is found in oranges (*Citrus Sinensis*), in flowers such as buckwheat (*Fagopyrum Esculent ump*), grapes nut (*Citrus para del*), and edible fruits.¹⁶² In hepatocytes, rutin more potentially increased Nrf2 expression and decrease nitric oxide synthase. Rutin also shows a hepatoprotective effect in opposition to CCl₄-initiates hepatic destruction in rats to prevent further hemodynamic changes which are related to ischemia and reperfusion through antioxidant activity.²⁰⁷

Luteolin

Luteolin is isolated from celery, thyme, and parsley³⁷ and is also found in many vegetables such as onion, broccoli, and cauliflowers.²⁰⁸ With the stimulation of PI3K/protein kinase B (Akt) and ERK1/2 signalling, luteolin also regulates the Nrf2/ARE pathway in HCC HepG2 cells, which further enhances the miRNA and nucleoprotein regulation of Nrf2 and HO-1.¹⁶⁰

CONCLUSION

Flavonoids have significant hepatoprotective potential, one of the key mechanisms by which they exert their effects is through the modulation of the Nrf2/ARE pathway. The Nrf2/ARE pathway plays a critical role in protecting liver cells from oxidative stress and inflammation, which are key factors in the development of liver diseases. Nuclear erythroid 2-related factor 2 (Nrf2) can activate cytoprotective genes and has a crucial role against oxidative stress to protect hepatic cells from oxidative damage. It has been identified that Nrf2 is also a prevailing factor in the regulation of ARE-mediated activation of other defensive genes, including GST, GCS, and HO-1. Flavonoids can stimulate the Nrf2/ARE pathway, by enhancing the expression of antioxidant and detoxifying enzymes and promoting liver cell survival and regeneration. This suggests that flavonoids may have therapeutic potential for the prevention and treatment of liver disease. However, more research is needed to fully understand the mechanism underlying the hepatoprotective effects of flavonoids and to identify specific flavonoids that may have clinical applications. Nevertheless, the promising finding suggests that flavonoids may represent a novel and effective approach to the prevention and treatment of liver diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Nrf2: Nuclear Factor erythroid 2-related Factor 2; **ROS:** Reactive oxygen species; **AMPK:** Adenosine monophosphate-activated protein kinase; **ARE:** Antioxidant response element; **JNK:** c-Jun N-terminal kinase; **MAPK:** Mitogen-activated protein kinase; **Neh:** Nrf2-ECH Homology; **NQO-1:NAD(P)H:** Quinone oxidoreductase-1; **PI3K:** Phosphatidylinositol-3-kinases; **sMAF:** Small musculoaponeurotic fibrosarcoma; **GST:** Glutathione-S transferase; **KEAP1:** KELCH-like ECH-associated protein; **GPx:** Glutathione peroxidase; **Akt:** Protein kinase B; **HCC:** Hepatocellular carcinoma; **ALD:** Alcoholic liver disease; **NASH:** Non-alcoholic fatty liver disease; **Trx:** Thioredoxin reductase; **AA:** Amino acids; **PERK:** Protein kinase-like ERK kinase; **HO-1:** Heme oxygenase-1; **SOD:** Superoxide dismutase; **ECM:** Extracellular matrix; **FXR:** Farnesoid X receptor; **CBP:** CREB-binding protein; **HSCs:** Hepatic stellate cells; **Rbx:** Ring box protein; **SIRT1:** Sirtuin 1; **TNF:** Tumoral necrosis factor-alpha; **UbcM2:** E2-ubiquitin-conjugating enzyme; **GCLC:** Glutamate-cysteine ligase catalytic subunit; **GSK3:** Glycogen synthase kinase 3; **GST:** Glutathione-S-transferase; **HBsAg:** Hepatitis B surface antigen; **HBx:** Proteins of HBV; **IHBs:** Intracellular hyaline bodies; **LXR:** Liver X receptor-alpha; **NASH:** Non-alcoholic steatohepatitis; **IL-1:** Interleukin-1; **NF-B:** nuclear factor kappa-B; **Bcl2:** B-cell lymphoma-2; **OS:** Oxidative stress; **PPARY:** Peroxisome proliferator activated receptor gamma; **SOD-1:** Superoxide dismutase-1; **SOD-2:** Superoxide dismutase-2; **SOD-3:** Superoxide dismutase-3; **LPS:** lipopolysaccharide; **CO:** Carbon monoxide; **Bax:** Bcl-2 associated X protein; **MCD:** Methionine-and choline-deficient; **HFD:** High-Fat diet; **αSMA:** Alpha-smooth muscle pain; **RNS:** Reactive nitrogen species; **ERK:** Extracellular signal-regulated kinase; **FOXO1:** Forkhead box protein O1; **MPKK:** Mitogen-activated Protein Kinase Kinase; **SREBP-1c:** Sterol regulatory element-binding protein-1c; **NLRP3:** Nucleotide-binding oligomerization domain-like receptor 3.

SUMMARY

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis, and 1 million due to viral hepatitis and hepatocellular carcinoma. Nuclear erythroid 2-related factor 2 (Nrf2) is a central regulator of antioxidative response elements-mediated gene expression. It has a significant role in adaptive responses to oxidative stress by interacting with the antioxidant response element, which induces the expression of various downstream targets aimed at cytoprotection. Flavonoid is among the most widely investigated herbal supplements for preventing liver illness. By triggering the normal ARE target affinity site, Nrf2 encourages the production of metabolic enzymes. It can also enhance the antioxidant and inflammation response and play a significant role in the illness. The present review briefly summarizes the mechanisms that

regulate the Nrf2/Keap1–ARE signaling pathway and the latest advances in understanding how flavonoid encourages the Nrf2 against oxidative stress with hepatoprotective effects. Further studies regarding the precise mechanisms of Nrf2-regulation are necessary for determining, whether flavonoid regulates Nrf2 and can serve as a therapeutic target in the treatment of hepatic disease.

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