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Dietary Antioxidants in Experimental Models of Liver Diseases

Sila Appak-Baskoy, Mustafa Cengiz, Ozgun Teksoy and Adnan Ayhanci

Abstract

Oxidative stress is caused by the imbalance between the amount of reactive oxygen species (ROS) and antioxidant capacity in the body. A balanced diet involving the daily intake of antioxidant-rich foods makes improvements in the total antioxidant capacity of individuals and would therefore reduce the incidence of oxidation-related diseases. It may also regulate the degree of oxidative stress. In fact, dietary micronutrients are either direct antioxidants or components of antioxidant enzymes, which may contribute positively to certain indicators of hepatic function. Liver plays an important role in the regulation of various processes such as metabolism, secretion, storage, and the clearance of endogenous and exogenous substances. Once liver is damaged by pursuing a wrong diet and inflammation takes place, most of these physiological functions get altered. Apart from drugs that used to treat the ailments, it is also necessary to determine the pharmaceutical alternatives for the drugs that are used in the treatment of liver diseases. Therefore, this chapter aims to summarize all known information on the effects of dietary nutrients on oxidative stress in experimental liver models.

Keywords: dietary antioxidant, free radicals, oxidative stress, liver

1. Introduction

The liver is the major organ metabolizing xenobiotics and endogenous molecules in order to maintain metabolic homeostasis in the organism, which is why it is a target of many toxic substances that cause dysregulated hepatic homeostasis. One of the mostly found clinical liver diseases is nonalcoholic fatty liver disease (NAFLD) (**Figure 1**) [2, 3]. In NAFLD, hepatocytes get filled up with triglycerides, liver expands and its normal functions may get altered. Although it is a disease, the triglyceride accumulation can still be reversed and normal functions may be restored by proper nutrition and exercise. If these lifestyle changes are not pursued, the damage goes on and results in inflammation followed with fibrosis which is unfortunately irreversible.

The liver is made up of hepatocytes, Kupffer cells, liver sinusoidal endothelial cells, pit cells, and hepatic stellate cells (HSC) [4]. Activation of stellate cells by injury caused by many etiological factors would lead to cirrhosis, and it would mark the end stage of progressive fibrosis [5]. Oxidative stress has a vital part in establishing

fibrosis and consequently cirrhosis [6]. For this reason, using molecules with antioxidant properties has been proposed as a treatment for not only fibrosis but also oxidative stress-related cirrhosis. Liver diseases are considered a major medical problem worldwide. There are known to be a large number of liver diseases caused by different insults. Furthermore, the disease type depends on lifestyle factors. For example, the main causes of liver diseases are reported to be viral and parasitic infections in regions like Africa and Asia. For Europe and America, alcohol consumption is thought to be the most important cause of this disease. However, viral hepatitis has showed an increase in recent times in most of the countries [7]. Lifestyle and unhealthy diet is the leading cause of liver diseases in almost all western countries. Until today, no medication is approved for the treatment of this disease; however, improving diet habits and physical exercise works if the disease is not accompanied by inflammation. On the other hand, biologically active food compounds that regulate gene expressions in lipogenesis, fibrosis, and inflammation serve as good therapeutic means to ameliorate these pathological states observed in liver [1] (**Figure 1**).

1.1 Oxidative stress

Oxidative stress is recognized as a disproportion between the production of free radicals (FR) and the antioxidant defenses [8]. Increased levels of prooxidants result in damage to the cell in terms of lipid peroxidation as well as oxidative DNA damage
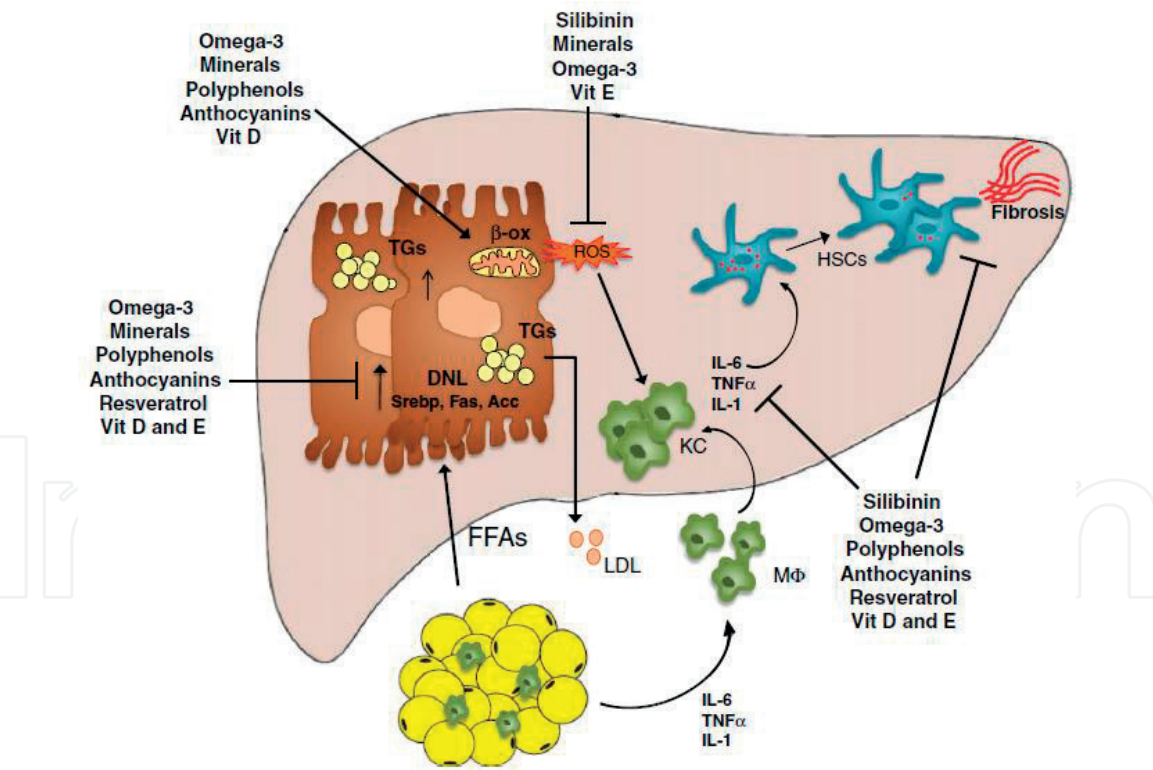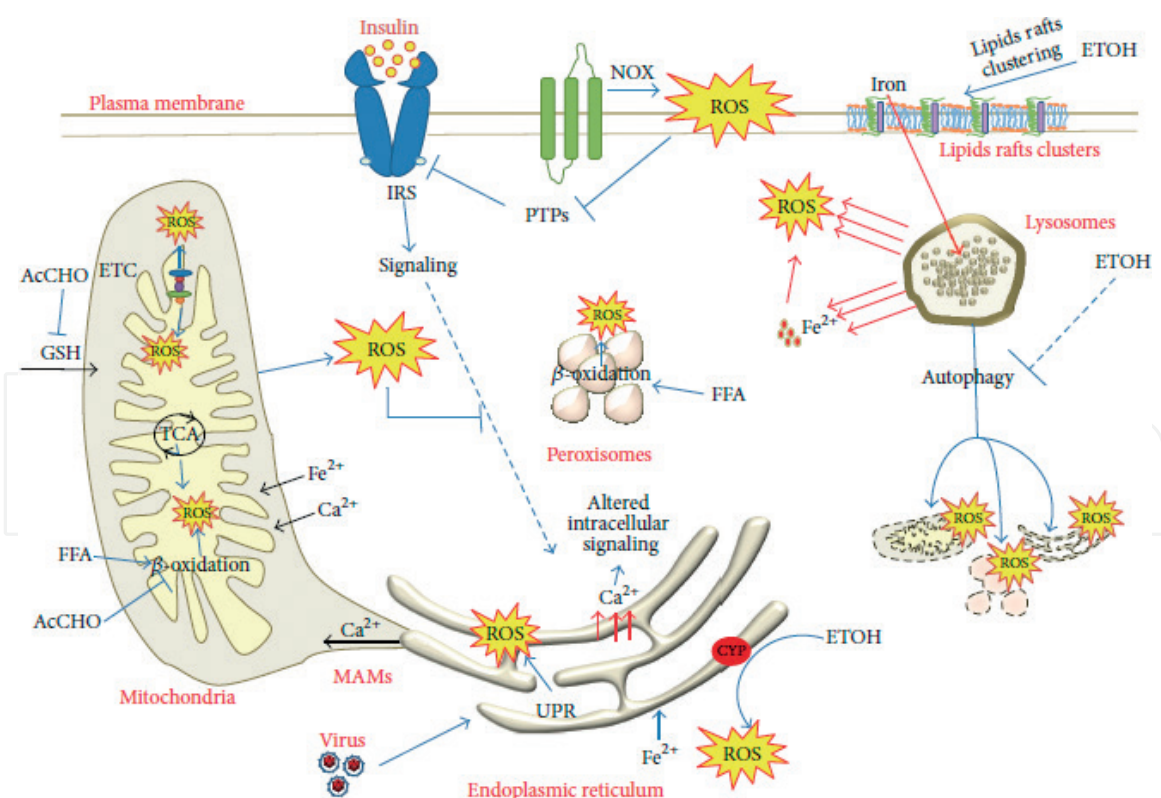

Figure 1.
Molecular mechanisms explaining the hepatoprotective effect of food bioactives. Development of NAFLD/NASH is induced by different risk factors, such as Western-type diet, physical inactivity, and genetic predisposition. In the presence of obesity and IR, there is an increased flux of FFAs to the liver. These FFAs are stored as TG in lipid droplets leading to hepatic fat accumulation or undergo β-oxidation increasing oxidative stress and the inflammatory pathway. The damaged hepatocyte leads to a further increase of inflammatory signaling (IL-1, TNFa, IL-6) and the recruitment of circulating and residual macrophages (KCs). All of these mechanisms can directly induce the activation of HSCs, the major cell type involved in extracellular matrix deposition and liver fibrosis. The bioactive compounds may exert beneficial effects on NAFLD development and progression by inhibiting lipogenesis, β-oxidation of FFAs, inflammation, and HSCs activation. In the cartoon, we have listed the food bioactives indicating the putative mechanisms by which they may improve liver damage in NAFLD [1].

and thus protein damage [9]. One or more unelectrified FR atoms or molecules may be present as radical cations or radical anions. They are usually unstable and highly reactive because they can react with molecules and abstract electrons. Oxygen can reduce and produce reactive oxygen species (ROS) with exciting electrons, secondary to the interaction of transition metals or by the addition of energy [9, 10]. Oxidative stress causes fibrogenesis by increasing transforming cytokines including transforming grown factor-beta-1 (TGFβ1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα) [3]. Disruption of the liver metabolism arises from increased quantity of ROS to amplified electron transfer in mitochondrial B-oxidation and increased expression and activity of Cyp2el that is from CYP450 family [11]. Intense production of Cyp2e1 is present because of much more consumption of ethanol which is produced by virtue of a lot of direct and indirect mechanisms [12–14] (**Figure 2**).

1.2 Basic sources of free radicals

Cells produce FR as a result of metabolic events; however, this is not the only source that can cause oxidative stress in body. The pollutants in the environment such as toxic chemicals as well as radiation cause a significant increase in amount of FR, ROS, and reactive nitrogen species (RNS) [10]. In the body, variety of different cell types and chemical reactions produce ROS, the most important metabolism is the cytochrome P450 metabolism and mitochondria-catalyzed electron transport reactions. Most of the inflammatory conditions are also responsible from ROS production, and important cell types in these processes are neutrophils, eosinophils,

Figure 2.
Mechanisms of enhanced ROS production during hepatocyte damage. Ethanol metabolism promotes strong ROS production in the ER by the inducible CYP. It impairs GSH import in the mitochondria, preventing ROS removal. It also impairs B-oxidation promoting lipid accumulation. ETOH induces lipid-raft clustering and increases iron uptake, promoting Fe^{2+} leakage from lysosomes and increased Fe^{2+} loads in mitochondria and ER, resulting in ROS production. Ethanol also reduced the autophagic removal of damaged cellular components. Viral infection challenges the ER protein folding process leading to ROS production and Ca^{2+} leakage in the cytosol and mitochondria. Increased MAMs formation promotes Ca^{2+} efflux from ER into mitochondria, increasing mitochondrial ROS production [15].

and macrophages [16, 17]. The chief molecule responsible for the reduction of oxygen in mitochondria is ubisemiquinone. Mitochondria is such an important organelle to produce ROS and hydrogen peroxide (H_2O_2) as it produces 2–3 nmol of superoxide/min per mg of protein, [17]. Different tissues of mammals and different species of mammals have an enzyme called xanthine oxidase, an enzyme belonging to molybdenum, iron-sulfur, flavin hydroxylases that play an important role in the hydroxylation of purines by the oxidation of hypoxanthine to xanthine. Resultant xanthine then oxidized to uric acid. Oxygen reduction takes place in both of these reactions and the first one produces O_2^-, while the second one produces H_2O_2 [16]. Inflammation serves as another source of ROS generation. During inflammation, activated macrophages increase their oxygen uptake, and this process results in production of O_2^-, nitric oxide (NO), and H_2O_2 [18]. Another mechanism of O_2^- production during inflammation is by neutrophils; the enzyme nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase generates O_2^- that is used to destroy bacteria and this nonphagocytic NAD(P)H oxidases produce O_2^- in a range of 1–10% [19]. Cytochrome P450 (CYP) enzymes are also important in the production of ROS by the breakdown and/or uncoupling of the P450 catalytic cycle. Hyperoxia would trigger 80% of the H_2O_2 synthesis by microsomes, and under normoxic conditions, peroxisomes produce H_2O_2 but not O_2^-, and most of the peroxisomal H_2O_2 production takes place in liver [16]. Arginine is reduced to citrulline in a five-electron oxidative reaction by nitric oxide synthases (NOSs) and this reaction gives rise to NO. Immune cells can also produce NO in the oxidative burst during inflammation. NO can react with oxygen and water in an extracellular environment in order to form nitrate and nitrite anions. Also, the NO and O_2^- can react together and cause a more reactive FR called peroxynitrite anion ($ONOO^-$) which can cause lipid peroxidation and fragmentation of DNA [20].

1.3 Antioxidants

Antioxidants are molecules that can help prevent or delay oxidation of an oxidizable substrate when in low concentrations and they have a high affinity to FR [21]. Antioxidants play an important role to maintain health of the organism by scavenging FR by donating electrons to it. This reduces the reactivity of FR and helps maintain prooxidant/antioxidant balance in cell. A lot of different molecules that have antioxidant activity have been identified. Different natural compounds have so far been studied extensively especially in liver diseases (**Table 1**).

1.4 Curcumin

Curcumin, diferuloylmethane or 1,7-bis (4-hydroxy-3-methoxyphenyl)1,6-hepadieno-3,5-dione is obtained from the rhizomes of *Curcuma longa* (turmeric). Curcumin has many pharmacological properties as it is a strong antioxidant, antifibrogenic, anti-inflammatory, antimicrobial, and anticarcinogenic agent and it also aids in a wound healing [22]. The Food and Drug Administration (FDA) has classified turmeric as a safe substance and toxicity assays done on animals have shown curcumin to be safe even when used in high doses. On the other hand, prolonged high-dose intake of turmeric has been associated with incidences of hepatotoxicity in mice and rats [3]. Curcumin is known to have low bioavailability when administered orally. Arcaro et al. [23] used piperine (inhibitor of hepatic and intestinal absorption) together with curcumin. Even in the presence of piperine, antidiabetic and antioxidant activity of curcumin was not altered. But when higher dose of piperine (40 mg/kg) was used, the beneficial effects of curcumin vanished. On the other hand, Sehgal et al. [24] showed the effect of piperine on curcumin in benzo(a)pyrene toxicity in the liver. They found that pretreatment with 100 mg/kg of curcumin protects against

Antioxidant	Main clinical effects	Clinical relevance
Curcumin	Antioxidant	No studies available in human hepatic disorders
	Antifibrotic	
	Anti-inflammatory	
	Antimicrobial	
	Wound healing	
	Anticarcinogenic	
Resveratrol	Antioxidant	Current data are conflicting, so more clinical studies are needed
	Anti-inflammatory	
	Anticarcinogenic	
	Lipid modulation	
	Antifibrotic	
Coffee	Antinecrotic	Inverse relationship between coffee-cirrhosis has been demonstrated, but more basic research and prospective clinical trials are necessary
	Antifibrotic	
	Antioxidant	
	Anticholestatic	
	Chemoprotective	
Quercetin	Chelation of transition metal ions	No studies available in human hepatic disorders
	Anticarcinogenic	
	Cardioprotective	
	Bacteriostatic	
	Antioxidant	
	Antifibrotic	
	Anti-inflammatory	
	Antiapoptotic	
	Antiaggregatory	
	Vasodilating	
Silymarin	Antioxidant	Silymarin has been shown to be effective, but it is necessary to do more clinical trials focused on survival rates of patients with cirrhosis
	Antifibrotic	
	Anti-inflammatory	
	Anticarcinogenic	
	Immunomodulation	
Naringenin	Antioxidant	No studies available in human hepatic disorders
	Hypocholesterolemic	
	Anti-estrogenic	
	Hypolipidemic	
	Antihypertensive	
	Anti-inflammatory	
	Antifibrotic	
	Anticarcinogenic	
	Antiatherogenic	

Antioxidant	Main clinical effects	Clinical relevance
Green tea	Anti-inflammatory	More clinical studies are needed
	Antiarthritic	
	Antimicrobial	
	Antioxidant	
	Neuroprotective	
	Antidiabetic	
	Antiangiogenesis	
	Anticarcinogenic	
L-carnitine	Chelation of transition metal ions	More clinical studies are needed
	Antioxidant	
	Cardioprotective	
	Neuroprotective	
	Anti-inflammatory	
Lycopene	Antioxidant	More clinical studies are needed
	Anti-inflammatory	
	Chemoprotective	
	Anticarcinogenic	

Table 1.
Nutritional antioxidants.

a single dose of benzo(a)pyrene; and at this dose, coadministration of piperine had a much better effect than did curcumin alone showing enhancer activity of piperine. In acute and chronic liver injury, curcumin has been shown to have hepatoprotective effects [25]. In 2007, Reyes-Gordillo et al. [26] showed that curcumin is able to inhibit the release of TNF-α, IL-1B, and IL-6. Additionally, curcumin reduces carbon tetrachloride ($CC1_4$)-mediated oxidative stress inactivating the nuclear factor-kB (NF-kB) pathway. Moreover, curcumin's hepatoprotective effect takes place by its interactions with Fe^{3+} and Cu^{2+}. A study by Jiao et al. [27] suggested that curcumin could serve as an iron chelator since transferrin receptor 1 and iron regulatory proteins, indicators of iron depletion, showed an increase with curcumin administration. Charoensuk et al. [28] have indicated that curcumin increases antioxidant capacity of cells by increasing mRNA and protein levels of factors and enzymes such as nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), glutamate cysteine ligase (GCL), transcription factor-3, peroxiredoxin 3 (Prdx3), and Prdx6. Curcumin also increases the activity of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) activity [29, 30]. Curcumin also interacts with enzymes or genes that are important in liver cirrhosis. Hassan et al. [31] showed that curcumin modulates miRNA 199 and 200 which are associated with liver fibrosis in $CC1_4$-induced experimental fibrosis model and that curcumin reduced these miRNAs levels close to their basal levels. Finally, in alcohol-induced liver damage, curcumin inhibits the activity of cytochrome P450 2E1 (Cyp2e1) and also its protein levels [32].

1.5 Resveratrol

The phytoalexin resveratrol (3,5,4′-trans-trihydroxystilbene) is a polyphenol mostly found in red grapes, red wine, peanuts, and berries [33]. Resveratrol has effects on lipid metabolism, and it also has antioxidant, anti-inflammatory,

anticarcinogenic, and antifibrogenic properties [34]. The rate of absorption of resveratrol is about 75% following an oral administration [35]. Resveratrol is metabolized to resveratrol sulfate, and in its low concentrations, it is converted into resveratrol glucuronide [36] by enzymes glucuronosyltransferase (UGT) or sulfotransferase (ST) [37]. In 2007, Chávez et al. [34] showed that under CCl_4, resveratrol decreased cytokine TGF-β levels and prevented hepatic fibrosis. It also inhibited NF-kB translocation to the nucleus. Resveratrol, as an antioxidant, has protective effects against ethanol-induced lipid peroxidation, toxicity by acetaminophen (APAP), and oxidative stress in animal models of cholestasis [38]. Important player in resveratrol's antioxidant activity is suggested to be run by the OH groups [7]. Blocking OH group methylation showed that resveratrol and trimethylated resveratrol provide some degree of protection, but the latter one has a better protective effect [39]. Another hepatoprotection mechanism of resveratrol comes from its ability to activate genes related to antioxidant system or from its ability to inhibit enzymes. A study by Cheng et al. [40] suggested that resveratrol could activate extracellular signal-regulated kinase (ERK) signaling pathway, which may, in turn, enhance the activation and translocation of Nrf2 to the nucleus, thus increasing the expression of HO-1 and glyoxalase. Price et al. [41] found that resveratrol activates AMP-activated protein kinase (AMPK) and increased nicotinamide adenine dinucleotide (NAD) levels in mice. Zhu et al. [42] have also shown that, in mice, administration of resveratrol increased the antioxidant system (SOD, GPx, and GSH) and also the levels of SIRTI and p-AMPK were upregulated in liver. Resveratrol has also been shown to inhibit the activity of Cyp2e1 in microsomes of rat liver [43]. Resveratrol also inhibited the activity of P450 isoform APAP-induced liver injury model [44] and activity of Cyp2e1 was also inhibited in diethylnitrosamine (DEN)-induced hepatocarcinogenesis model [45]. The only clinical study that was performed to determine the resveratrol hepatoprotective effect demonstrated that a 500 mg resveratrol dose administrated for 12 weeks caused a significant reduction in inflammatory cytokines, serum cytokeratin-18, NF-kB activation, liver alanine aminotransferase (ALT), and hepatic steatosis when compared to the placebo group in patients with nonalcoholic fatty liver disease (NAFLD) [46].

1.6 Coffee

Coffee is a mixture of several different molecules such as carbohydrates, vitamins, lipids, nitrogenous molecules, alkaloids, and phenolic compounds [47]. Caffeine, diterpene alcohols (cafestol and kahweol), and chlorogenic acid are the three major compounds found in coffee [48]. Coffee consumption has been linked to the reduction of several chronic diseases [49], probably due to the pharmacological properties that have antinecrotic, antifibrotic, anticholestatic, chemoprotective, and antioxidant functions [50]. Caffeine is the best-known active component of coffee, which is absorbed very rapidly once it has been taken orally (5 min), reaching its peak blood levels after 30 min. When consumed in high amounts, it may produce some side effects. Recommendations from Health Canada in 2013 demanded that the daily caffeine intake for children should not exceed 2.5 mg/kg of the body weight. What is more, tachycardia and arrhythmia typically arise when more than 200 mg of caffeine are ingested [51]. Smith et al. [52] reported in 2002 that the intake of 300 mg of caffeine resulted in a rise in anxiety and tension. Caffeine gets metabolized in the liver. The principal metabolite of caffeine is paraxanthine [53]. An important property of caffeine is that it can easily pass through the blood-brain barrier [54]. Coffee-cirrhosis relationship was shown by Klatsky et al. for the first time [55]. The study showed that the odds ratio for liver cirrhosis tend to decrease from 1.0 for people abstaining from coffee to 0.47, 0.23, 0.21, and 0.16 for 1, 2, 3, or 4 cups of coffee daily, respectively. Although coffee is generally beneficial to the liver, this study failed to show a causative

role of coffee in prevention of liver injury. Therefore, additional basic research and controlled prospective studies are needed in order to show exact effect of coffee on liver tissue. Arauz et al. [50] demonstrated that coffee has a protective effect upon liver injury caused by chronic administration of thioacetamide (TAA). Coffee ameliorated cholestasis and necrosis and this was seen by the measurement of γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase, and ALT levels. Arauz et al. [50] demonstrated in murine models that coffee prevents experimental liver cirrhosis. In these studies, coffee reduced the expression of the profibrogenic cytokine TGF-β. Cavin et al. [56] reported coffee to be an inductor of GST, aldo-keto reductase, GSH, HO-1, GST-P1, which are enzymes involved in the detoxification process. Also, they suggested that a possible mechanism of chemoprotection of coffee by stimulating the Nrf2 pathway. In another study, coffee was able to elevate mRNA levels of NQO1 and glutathione-S transferase Al in the liver and the small intestine [57].

1.7 Quercetin

Quercetin, 3,3,4,5,7-penta-hydroxyflavone, is a flavonol especially found in apples and onions [58]. Quercetin chelates heavy metals and has anticarcinogenic, cardioprotective, bacteriostatic, anti-inflammatory, and antioxidant properties [59]; it also functions as a hepatoprotective agent [60]. The normal daily intake of quercetin is less than 5–40 mg. However, if the peels of the foods that contain high amount of quercetin are also consumed, daily intake of quercetin increases to 200–500 mg [59]. In 2004, high-purity quercetin used in foods was grass that serves 10–125 mg of quercetin [59]. The functional groups responsible for quercetin's antioxidant activity were described by Bors et al. [61] in 1990, and they found that orthodihydroxy or catechol groups in the B-ring, a 2,3-double bond of the C-ring, and OH substitution on positions 3 and 5 of the C-ring and A-ring, respectively, are important players in antioxidant action of quercetin [61]. It can interact with both FR and metal ions like Fe^{3+} and Cu^{2+} for chelation. In a study by Mira et al. [62], it was reported that reduction of Fe^{3+} and Cu^{2+} takes place by quercetin's 2,3-double bond and the presence of catechol group in the B-ring. Following ingestion, quercetin is rapidly absorbed and its levels in blood peak at approximately in 30 min [63] before it is metabolized by glucuronidation and sulfation by the UGT and ST, respectively.

In experimental fibrosis model in rats, quercetin showed hepatoprotective properties under CCl_4 treatment that lasted 8 weeks. The hepatoprotective effect of quercetin was found to be mediated by its ability to suppress the expression of profibrogenic expressions of TGF-β, CTGF, and collagen-lα (Col-1α). On the other hand, quercetin also activated enzymes such as metalloproteinases 2 and 9 (MMP2 and MMP9); it also improved the activity of SOD and CAT [64]. Pavanato et al. [65] extended CCl_4 treatment for 16 weeks and also observed that quercetin improved the hepatic liver enzymes AST, ALT, and inducible NOS (INOS) expressions; it also decreased collagen amount and reduced lipid peroxidation in liver. Granado-Serrano et al. [66] showed that quercetin modulated Nrf2 and p38 in HepG2 cells. Quercetin has also been shown to suppress the activity of Cyp2e1 in hepatocytes in the presence of ethanol [67]. In line with this finding, in a nonalcoholic steatohepatitis (NASH) model, quercetin was able to reduce Cyp2el activity [68].
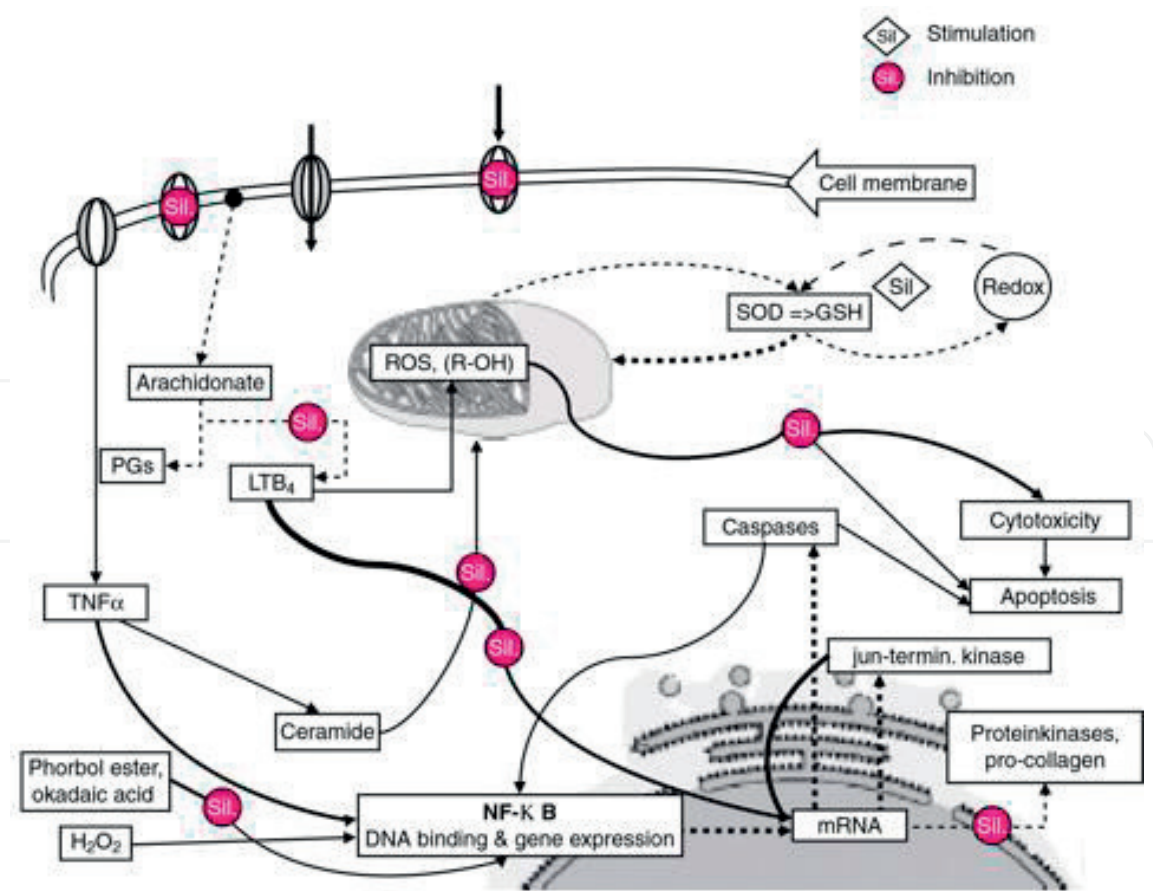
1.8 Silymarin

Silymarin, milk thistle or Saint Mary's thistle, is a natural substance obtained from *Silybum marianum* [7]. Silymarin has not been associated with any side effects at acute consumption and the dose range used in literature ranges between 280 and 800 mg/kg of body weight per day. After oral administration, the silymarin peak

plasma concentration is achieved at approximately 6–8 h. The metabolites of silymarin get conjugated in the liver by UGT and ST (phase II reactions) [69]. This substance has many hepatoprotective effects (**Figure 3**). In fact, silybin, a major constituent of silymarin, has found to have iron chelating properties [71]. In a study performed by Najafzadeh et al. [72], hepatoprotective effect of silymarin in iron-overload-induced hepatotoxicity was attributed to its iron-chelator activity; however, no studies have proved the chelating properties per se of silymarin in liver diseases. Silymarin acts as hepatoprotector against several hepatotoxins including D-galactosamine [73]. Silymarin's ameliorating effects on oxidative stress, fibrosis, cirrhosis, and lipid peroxidation are modulated by its phosphatidylethanolamine amount [74]. This hepatoprotective effect is seen with the improvement of liver enzyme activities and levels of cholesterol/phospholipids and also sphingomyelin/phosphatidylcholine ratios in the membrane [75, 76]. Kim et al. [77] showed that silymarin increases nuclear translocation of Nrf2 in activated HSC. Also, silymarin increases the activity of antioxidant enzymes such as SOD, GPX [78], and CAT [79]. A clinical trial examining silymarin in a complex with phosphatidylcholine found reduced levels of the liver enzymes, ALT and γ-GGT, and serum bilirubin levels in a dose-dependent manner in patients suffering from hepatitis due to virus infection or alcohol abuse [80] (**Figure 3**).

1.9 Naringenin

Naringenin is also recognized as 5,7,4′-thihydroxyflavanone, and it is a flavanone found in citrus fruits and tomatoes [81]. In a recent study, Yang et al. [82] have

Figure 3.
A schematic notation of the main pharmacological effects of silymarin in accordance with its hepatoprotective features: The effects of Sm upon cell membranes (upper left) and intracellular cascades are shown here. The metabolic paths are indicated by interrupted lines, while its signal effects are shown in full lines. LTB4, leukotriene B4; GSH, glutathione; NF-kB, nuclear factor kappa B; PG's, prostaglandins; Sm, silymarin; SOD, superoxide dismutase; ROS, reactive oxygen types=; TNFa, tumor necrosis factor α [70].

reported that naringenin did not cause any harmful effects in beagle dogs, the maximum time of exposure being 180 days and with doses varying of 20, 100, or 500 mg/kg body weight per day. Naringenin has many pharmacological properties. It acts as a hypolipidemic, antihypertensive, anti-inflammatory, antioxidant, and antifibrotic agent [81]. The metabolism of naringenin takes place in small intestine where glycoside form of naringenin gets cleaved, resulting in sulfate and glucuronide metabolites in the small intestine wall; then, it gets absorbed [77]. Mira et al. [62] showed that naringenin can reduce Fe^{3+} and Cu^{2+} ions but it is less potent than quercetin. Chtourou et al. [83] found that naringenin averts depletion of SOD, CAT, GPx, and GSH. On the other hand, naringenin also prevents an increase in lipid peroxidation, and it also prevents increase of enzymes ALT and AST [78]. Yen et al. [84] also obtained similar results on liver enzymes and prevention of lipid peroxidation when they used naringenin alone and also naringenin-loaded nanoparticle system (NARN). In both treatments, naringenin exhibited antioxidant and hepatoprotective activities. In these experiments, treatment with naringenin also inhibited the activation of caspases 3 and 8. However, NARN was found to have better hepatoprotective and antioxidant effects than free naringenin, and it was also shown to inhibit caspase 9 during $CC1_4$-induced hepatotoxicity in rats. Han et al. [64] reported that a pretreatment with naringenin-7-O-glucoside increases NQO1 and ERK phosphorylation and translocation of Nrf2 to the nucleus in H9c2 cardiomyocytes. It also upregulated the mRNA expression of GCLC and GCL modifier [64]. Similar findings have been reported by Esmaeili et al. [85] who showed that naringenin attenuates $CC1_4$-induced liver injury by downregulating TNF-α, INOS, and cyclooxigenase-2 and also by increasing Nfr2 and HO-1 expressions. Motawi et al. [86] showed that naringenin inhibits Cyp2e1 in liver microsomal assay done on rats [86].

1.10 Green tea

Camellia sinensis or green tea is a widely consumed beverage across the globe and it has antioxidant, anti-inflammatory, antiarthritic, and antiangiogenic effects. It is a mixture of polyphenols (the major class of active compounds) including catechins (also known as flavan-3-ols) which constitute about 30% (mass fraction) of green tea leaves. The major catechins in green tea are (+) -catechin, (−) -epicatechin, (−) -epigallocatechin, (−) -epicatechin-3-gallate, (−) -gallocatechin, (−) -gallocatechin gallate, and (−) -epigallocatechin-3-gallate (EGCG). EGCG is the most abundant catechin accounting for 50% of total polyphenols; thus, it is the main biological active compound of green tea [87]. However, polyphenols are not the only compounds that green tea exerts its antioxidant activity with through. The amino acid, L-theanine, in green tea accounts for 1–2% of the leaf dry weight that is synthetized in the roots of green tea and is concentrated in the leaves. Studies have reported that L-theanine protects the cell by maintaining its GSH levels in cancer and neurotoxic diseases [88]. The intake of green tea can be considered safe unless its consumption exceeds 1–2 cups a day. And higher consumption such as in attempts to lose weight resulted in hepatotoxicity [87]. At normal doses, Pérez-Vargas et al. [88] found that the main amino acid of green tea, L-theanine, reduced expression of NF-kB and downregulated IL-1β and IL-6 and the cytokines TGF-β and CTGF. Halegoua-De Marzio et al. [89] tested a single high dose of green tea (400 mg), in patients with HCV-induced cirrhosis and found that it is well tolerated by patients and beneficial for treating cirrhosis.
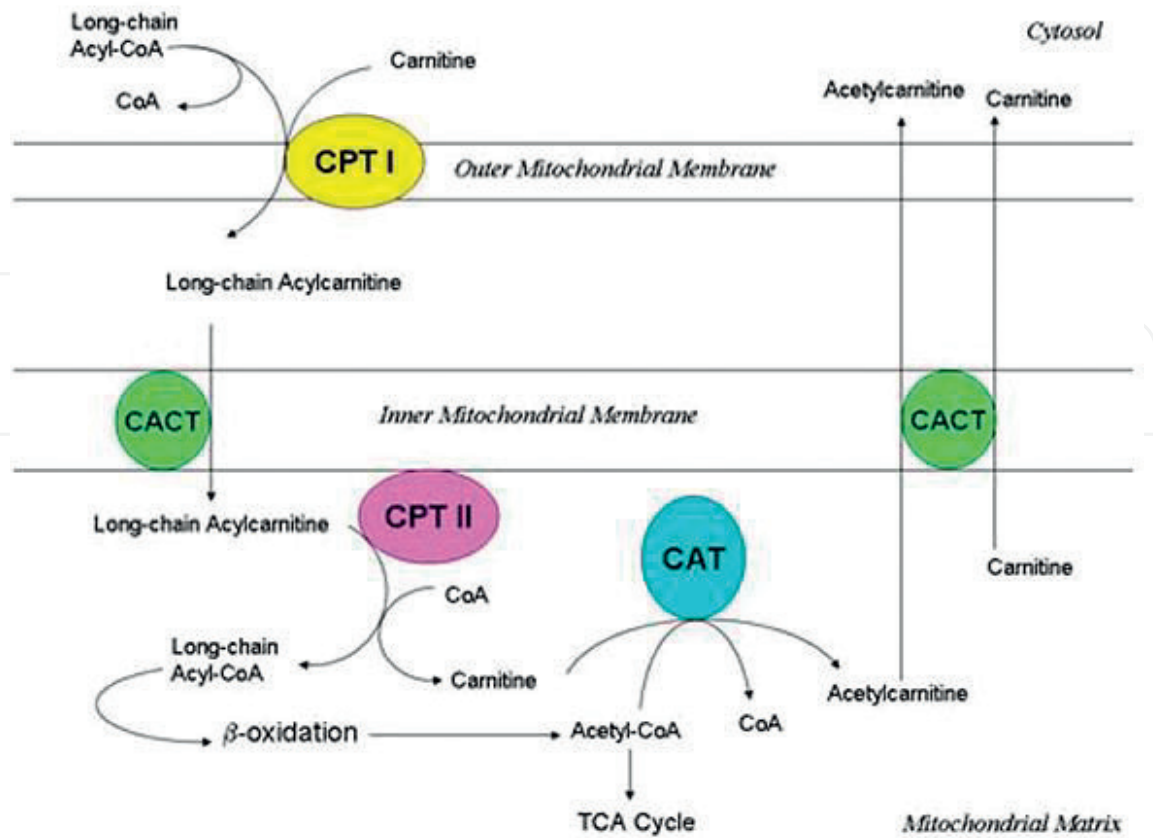
1.11 L-Carnitine

L-Carnitine (LC), B-hydroxy-y-trimethylaminobutyric acid, is a water-soluble molecule important in mitochondrial oxidation of fatty acids in mammalian

metabolism (**Figure 4**). LC can exist in three different forms: as free LC, acetyl-L-carnitine (ALC), or other carnitine esters. About 25% of carnitine is obtained from methionine biosynthesis, but most LC is provided by the diet, especially through red meat and milk consumption [91]. LC acts as a carrier of fatty acids across the inner mitochondrial membrane for β-oxidation and ATP production. Apart from its role in the lipid metabolism, LC is also a potent antioxidant, and it protects tissues from oxidative damage. Reduced concentrations of LC in the body are mostly due to the accumulating toxic metabolites and also because of lack of protein in restricted diets. Thus, LC supplementation could be useful not only to supply the tissues in presence of but also in avoiding oxidative damage as a result of increased amounts of reactive species. Since LC can easily cross the blood-brain barrier, LC supplementation may also be beneficial in preventing oxidative injury-related neurological damage and further studies are needed in order to clearly establish LC's role in neurological diseases [92].

1.12 Lycopene

Lycopene (LYC) is an acyclic isomer of beta-carotene which has great antioxidant activities. It is synthesized by plants or autotrophic bacteria but not by animals. Red fruits and vegetables, such as tomatoes, watermelons, pink grapefruits, apricots, pink guavas, and papaya, contain LYC. Studies show that LYC consumption not only reduces the risk of cancer of many organs but also retards the growth of tumors. LYC has been shown to have protective effects on other pathologies such as cardiovascular diseases, osteoporosis, male infertility, and this action is mainly mediated by LC's ability to inhibit other toxic agents (**Figure 5**). Numerous *in vitro*

Figure 4.
The mitochondrial carnitine system. Abbreviations: CPT I, carnitine-palmitoyl transferase I; CACT, carnitine acyl carnitine transferase; CPT II, carnitine-palmitoyl transferase II; CAT, carnitine acyl transferase; CoA, coenzyme A [90].

and *in vivo* studies showed that LYC could provide protection against ionizing radiation. Therefore, supplementation of LYC might be protective against damaging effects of radiotherapy in cancer treatments and it can also be protective against accidental radiation exposure [94].

1.13 Piperine

Piperine [1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4, pentadienyl] piperidine] is the major pungent alkaloid present in the fruits of *Piper nigrum* L. [95]. Piperine at low concentrations acts as a hydroxyl radical scavenger, but at higher concentrations, it activates the Fenton reaction, resulting in increased generation of hydroxyl radicals. Piperine has hepatoprotective effects and it was shown to inhibit lipid peroxidation in the rat liver microsomes at a concentration of 600 μM [96].

1.14 Capsaicin

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the major strong and irritating ingredient of red pepper. It may inhibit copper ion-induced lipid peroxidation of human LDL, which suggests that it is an effective antioxidant offering protection against oxidation of human LDL [97].
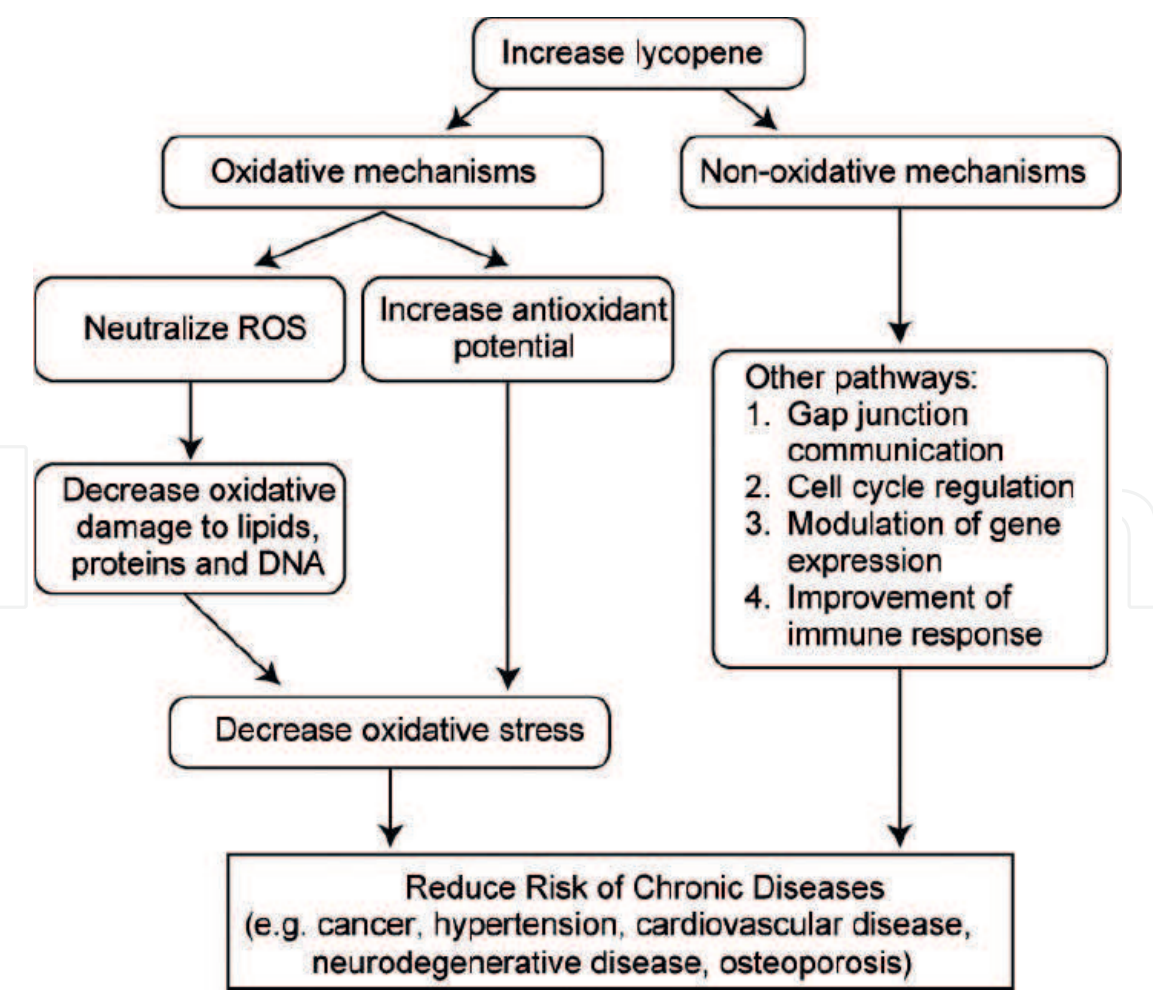
Figure 5.
General mechanisms of action of lycopene. The proposed mechanisms of action of lycopene (oxidative and nonoxidative) that decreases the risk of oxidative stress-mediated diseases. Lycopene most likely acts via the oxidative mechanism of action to prevent oxidative stress and its detrimental effects on male infertility. ROS: reactive oxygen species [93].

1.15 Garlic and onion

Diallyl sulfides and diallyl disulfides, the active components of garlic, have anti-inflammatory and antimutagenic activities. Onion is a major source of flavonoids, especially the two quercetin glycosides, quercetin 4-*o*-*β*-glucoside and quercetin 3,4-*o*-*β*-diglucosides, which are recognized as bioactive substances. In order to show the antioxidant properties and protective effects of garlic and onion, a study was carried out on rats. Animals were treated with 0.6 mg nicotine/kg and also given 100 mg garlic or onion oils/kg for 21 days. Nicotine increased concentrations of thiobarbituric acid, conjugated dienes, and hydroperoxides in the tissues. Supplementation with both the garlic oil and onion oil increased resistance not only to lipid peroxidation but they also increased levels of antioxidant enzymes and glutathione. These conclusions state that oils of garlic and onion are effective antioxidants against nicotine-related oxidative stress and damage [98].

1.16 Vitamins C and E

Vitamin C, substrate for ascorbate peroxidase, is not only a highly effective antioxidant but also an essential component of a healthy diet. Vitamin E, the major antioxidant found in lipid composition of membranes, is a fat-soluble antioxidant. During fat oxidization, vitamin E helps to inhibit formation of ROS [99]. Several studies showed that vitamin E serum levels are significantly reduced in alcoholic liver disease [100]. It is also shown that vitamin E levels are inversely proportional to formation of oxidative stress products that correlate with the extent of liver damage [101]. For this reason, maintenance of normal concentrations of vitamin E appears to be necessary for preventing lipid peroxidation due to alcohol consumption. Works from several laboratories have so far indicated that mitochondrial damage may present a common early event in cell injury [102]. It is possible to prevent mitochondrial damage through vitamin E [103]. Vitamin E or C alone, or in combination, can ease scavenging free radicals that are generated in the liver tissue [104]. In the mouse model, vitamin E supplementation restores alcohol-induced redox status, reduces apoptosis, and prevents oxidative stress [105]. What is more, vitamin E is effective in doses of 600 mg daily when it comes to suppressing HBV replication and normalizing ALT in a significant proportion of chronically infected patients with CLD [106].

1.17 Trace minerals

Trace minerals act as a cofactor of antioxidant enzymes thus enabling the antioxidant activities to take place. These trace minerals include selenium (Se), zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) [103]. O_2^- radicals are eliminated by the enzyme Cu-Zn-SOD and Cu and Zn are the co-factors for the enzyme. One of the enzymes responsible for H_2O_2 clearance from the cells is CAT and Fe is the essential cofactor of this enzyme. Levels of ferritin may decline with exercise and increasing dietary or supplemental Fe can improve performance. It was shown that moderate-level supplementation of Fe to competitive swimmers increased their performance and helped to maintain normal ferritin levels [107]. Selenium (Se) is a cofactor for the antioxidant enzyme GPx, which is like the enzyme CAT, responsible for removing H_2O_2 and other organic H_2O_2 from the cell. A study by Akil et al. [108] showed in rats, that upon acute swimming exercise, lipid peroxidation in the brain was increased and Se supplementation to these rats increased antioxidant activity resulting in inhibition of the free radical production [108]. Manganese (Mn) is a cofactor for the enzyme Mn-SOD. It eliminates O_2^- radicals produced during oxidative phosphorylation [109].

2. Conclusion

Investigations done on antioxidants have shown that these compounds are candidates for the treatment and candidates to prevent oxidative stress-related diseases. This chapter focuses on antioxidants that can be investigated in experimental and clinical trials of many diseases but especially in diseases of liver. Main nutritional components involved in the production and/or removal of free radicals and the role of free radicals in the pathogenesis of several hepatic diseases and related comorbidities have been described in this chapter.

Among the antioxidants that were described, curcumin, naringenin, and quercetin have been found to be effective antioxidants in treatment of experimental liver injury. Green tea has been shown to protect against different kinds of cancer in clinical trials but not on hepatocellular carcinoma. Resveratrol has been extensively studied in experimental models of liver diseases and has been shown to have protective effects on fibrosis. So far, there are not much clinical trials on ameliorating and disease preventing effects of most potent antioxidants on liver and these antioxidants are good candidates for clinical trials not only because they show great disease preventing and ameliorating effects but also because they are derived from food sources and have a good metabolic tolerance.

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