OXIDATIVE STRESS AND CHRONIC ALCOHOL LIVER DISEASE: THE CURRENT PERSPECTIVES

Ms. Mamta Singh¹, Dr. Seema Gupta², Dr. Rajesh Pandey³, Dr. H.K. Aggarwal⁴, Dr. S. K. Aggarwal⁵

¹Research scholar, Department of Biochemistry, NIMS Medical College, NIMS University, Jaipur, India
²Assistant Professor, Department of Biochemistry, Government Medical College, Haldwani, Uttarakhand, India
³Associate Professor, Department of Biochemistry, M. M. Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India
⁴Professor, Department of Medicine, PGIMS Rohtak, Haryana, India
⁵Professor, Department of Biochemistry, M. M. Medical College and Hospital, M. M. University, Kumarhatti, Solan, H.P. India

ABSTRACT

Alcohol liver disease (ALD) is characterized by genetic, psycho-social and environmental attributes that influence its development and manifestations. The disease is often progressive and is considered to be a major cause of morbidity and mortality. Alcohol, the most important hepatotoxin, can injure the liver cells directly and/or through its toxic metabolites, i.e. acetaldehyde and free radicals. Besides promoting the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), long term ethanol ingestion might deplete the hepatocytes of some important secondary components of the antioxidant defence system (enzymatic and non-enzymatic). Thus, alcohol and its metabolites tip the balance in favour of sustained oxidative stress which seems to be is one of the important biochemical manifestations of ALD. It is therefore, worthwhile to evaluate the degree of oxidative stress and the front line component of antioxidant defense system in patients suffering from ALD. Current research is contributed in a better understanding for the role of oxidative stress in context of the disease severity to augment the antioxidant defence system in the supportive management of ALD. Antioxidant’s administration is a good therapeutic strategy for the treatment of ALD and suggests the regular screening of antioxidant levels should be done to know the deficient or the excess status.

Please cite this article in press as Ms Mamta et al. Oxidative Stress and Chronic Alcohol Liver Disease: the Current Perspectives. Indo American Journal of Pharm Research.2014;4(03).
INTRODUCTION

Alcoholism is called a “dual disease” since it includes both mental and physical components. The Social environment, stress, mental health, family history, age, ethnic group and male, all influence the risk for condition [1]. Alcohol is consumed at some time in their life by 80% of the population [2]. Alcohol affects all systems ranging from central nervous system, cardio-vascular system and genitourinary system but the liver most. The alcoholic liver disease encompasses a spectrum of injury, ranging from simple steatosis to frank cirrhosis [3].

Alcohol mediated increased production of pro-oxidants as well as decrease in the antioxidant defense systems (enzymatic and non-enzymatic) in liver may contribute to the development of ALD. An imbalance between pro-oxidants and antioxidants leads to oxidative stress, characterized by escalating cell damage. Acetaldehyde, an immediate metabolic product of alcohol oxidation, has been implicated in the generation of neutrophils chemo-attractants and the activated tissue neutrophils further contribute in tissue oxidative injury by releasing O$_2^-$ [4]. It is therefore, worthwhile to evaluate the degree of oxidative stress and the front line component of antioxidant defense system in patients suffering from ALD. The free radicals, generated during alcohol metabolism are highly reactive molecular fragments that frequently contain oxygen and cause much damage to the cells. These free radicals impair the antioxidant defaces of the body and interact destructively with vital cell constituents, potentially causing cell death.

Inadequate removal of ROS may cause cell damage by attacking membrane lipids, proteins and inactivating enzymes thus mediating several forms of tissue damage. A variety of enzymatic and non enzymatic mechanisms have evolved to protect cells against ROS. An appropriate balance of enzymatic and non-enzymatic antioxidant defence is necessary for withstanding the destruction caused by the ROS and reduce the damage caused by oxidative stress due to alcohol in ALD.

Epidemiology and prevalence of alcohol liver disease (ALD)

Alcohol remains a major cause of liver disease worldwide and is a most frequent cause of liver disease in the advanced countries [5]. The past two to three decades have seen stabilization if not a drop in the intake of alcohol in the western countries while a very adverse trend is reported from Eastern Europe and the developing countries [6]. Alcohol liver disease accounts for >50% of all chronic liver diseases in industrialized countries and is responsible for >50 000 annual deaths due to cirrhosis and associated complications [7, 8].

ALD comprises various degrees of liver injury due to direct and indirect effects of continuous exposure towards toxic amounts of alcohol, including alcoholic fatty liver, alcoholic steato-hepatitis, as well as alcohol-induced hepatic fibrosis and cirrhosis, either with or without inflammation [9]. Moreover, chronic alcohol consumption is an established risk factor for the development of hepatocellular carcinoma in patients with liver cirrhosis [10]. While nearly all heavy drinkers reveal fatty liver, 10–35% of alcoholics are diagnosed with alcoholic hepatitis, and 10–20% develops cirrhosis [11]. Hence, only a minority of heavy drinkers develop severe liver disease. Although necessary, excessive alcohol use is not sufficient to promote ALD. Only 1 in 5 heavy drinkers develops alcoholic hepatitis, and 1 in 4 develops cirrhosis [12].

The prevalence of ALD influenced by many factors, including genetic factors (e.g., predilection to alcohol abuse, gender) and environmental factors (e.g., availability of alcohol, social acceptability of alcohol use, concomitant hepatotoxic insults), and it is therefore difficult to define. Failure to recognize alcoholism remains a significant problem and impairs efforts at both the prevention and the management of patients with ALD [13]. Although the exact prevalence is unknown, approximately 7.4% of adult Americans were estimated to meet the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, criteria for the diagnosis of alcohol abuse and /or alcohol dependence in 1994; [14] more recent data suggest 4.65% meet the criteria for alcohol abuse and 3.81% for alcohol dependence [15]. In 2003, 44% of all deaths from liver disease were attributed to alcohol [16]. Although there are limitations of the available data, the World Health Organization’s Global Alcohol database, which has been in existence since 1996, has been used to estimate the worldwide patterns of alcohol consumption and allow comparisons of alcohol-related morbidity and mortality [17].

Risk factors for Alcohol Liver Disease (ALD)

The individual susceptibility to develop ALD depends on genetic and nutritional factors. The disease process is characterized by early steatosis, inflammation and cirrhosis under the influence of various factors as shown in Figure 1.

The individual susceptibility to develop ALD depends on genetic and nutritional factors. The disease process is characterized by early steatosis, inflammation and cirrhosis under the influence of various factors as shown in Figure 1. There are many factors which help in development of ALD. The most important factor is amount of alcohol ingested [18]. While the relationship between amount and pathogenesis of ALD is not clear but a significant correlation exists between per capita consumption and the prevalence of cirrhosis. The risk of developing liver disease is also influenced by type of alcohol. In Denmark, drinking beer or spirits was more likely to be associated with liver disease than drinking wine [19]. Another factor that is associated with ALD is drinking pattern like in comparison between drinking at meal time with drinking exterior of meal times has been reported to increase the risk of ALD by 2.7-fold [20]. Binge drinking, also been shown to increase the risk of ALD and all-cause mortality [21]. Lack of nutrients in diet leads to protein energy malnutrition in ALD patients.
Ms Mamta, et. al.

Spectrum of Alcoholic Liver Disease (ALD)

The spectrum of liver damage caused by alcohol is not uniform. For descriptive purposes, three main histological stages are described as if they constitute separate and definitive lesions: steatosis, acute alcoholic hepatitis and cirrhosis [30]. In reality, these entities overlap, and it is difficult to find them isolated in their pure histo-pathological form. Steatosis is a predictable histological abnormality which develops in many heavy drinkers. It results from the redox imbalance generated by the metabolism of ethanol to acetate. Alcoholic steatosis completely reverses within several weeks of discontinuation of alcohol intake [31].

Acute alcoholic hepatitis is characterized by hepatocellular injury with associated inflammation and fibrosis. Like steatosis, acute alcoholic hepatitis usually improves with abstinence. When alcohol use continues unabated, inflammation triggers fibrogenesis and, over time, collagen is deposited in a characteristic perivenular and pericellular distribution. Approximately 40% of patients with this lesion (zone 3 fibrosis extending in a lattice-like peri-hepatocyte network) will develop cirrhosis within 5 years [32]. Severe acute alcoholic hepatitis has a poor outcome with standard supportive management. For example, the mortality rate of patients with severe alcoholic hepatitis in two prospective studies was 35% and 46%, respectively [33]. The addition of acute renal failure worsens the prognosis further [34].

Metabolism of Alcohol

Alcohol is absorbed rapidly in the gastrointestinal tract; the surface of greatest adsorption is the first portion of the small intestine with 70%; 20% is absorbed in the stomach, and the remainder, in the colon. Diverse factors can cause the increase in...
absorption speed, such as gastric emptying, ingestion without food, ethanol dilution (maximum absorption occurs at a 20% concentration), and carbonation. Under optimal conditions, 80–90% of the ingested dose is completely absorbed within 60 minutes. Alcohol has a high affinity for water and is therefore found in body tissues and fluids inasmuch as they contain water. Once ethanol is absorbed, it is rapidly carried throughout the body in the blood and once absorption of alcohol is complete equilibrium occurs such that blood at all points in the system contains approximately the same concentration of alcohol. Alcohol is eliminated mainly (> 90%) by the liver through the enzymatic oxidation pathway; 5-10% is excreted without changes by the kidneys, lungs, and in sweat. Alcohol metabolism is take place in liver by two pathways i.e., oxidative pathways (through pathways involving ADH, cytochrome P450, and catalase enzymes), and non-oxidative pathways as shown in Figure 2.

![Figure 2: Pathways of alcohol metabolism.](image)

The alcohol metabolism through the following three different enzymatic systems: with the enzyme alcohol dehydrogenase (ADH), with cytochrome P-4502E1 (CYP2E1) and with mitochondrial catalase. Only the first two are of the practical significance - ADH finds use in the degradation of relatively small quantities of alcohol, alcohol-induced CYP2E1-in excessive alcohol intake. Apart from the liver, ADH is also present in the gastric mucosa, and the assumption is that individuals with low gastric ADH activity are more susceptible to ALD. This may also help to explain why women who have decreased gastric ADH activity [35] are more prone to developing ALD. Both enzymes convert alcohol to acetaldehyde which is in part responsible for the hepatotoxic damage.

However, the liver damage process is much more complex, it results from biochemical, genetic, cellular, immunologic and humoral disorders in connection with the intake and metabolism of excessive quantities of alcohol [36]. A major role has the oxidative stress which results mainly from alcohol-induced CYP2E1, by simultaneous shortage of antioxidants in the hepatocytes, but also by damage caused by acetaldehyde alone and altered balance of many cytokines- mainly TNF-α [37]. Changes in the lipid metabolism and in adipose tissue also contribute to the process [38].

**Metabolic alterations associated with Alcohol Metabolism**

Since the major parts of alcohol metabolism take place in it, the liver is an organ that is especially susceptible to toxic effects from metabolites of alcohol. The different pathways of alcohol metabolism described above have numerous detrimental consequences that contribute to the tissue damage and diseases seen in alcoholic patients. The various harmful compounds (adducts, ROS, RNS) are generated due to alcohol metabolism by products and other cell component’s interactions which lead to change in the ratio of NADH to NAD⁺ (i.e., the cell’s redox state). This shift is due to oxidation of alcohol in the liver. These consequences and the way they contribute to tissue damage and disease will be discussed as follow:
1. The high NADH level in cytoplasm favors the conversion of pyruvate to lactate, enhanced lactate production and accumulation leads to lactic acidosis. There is decrease in the availability of pyruvate for oxaloacetate generation couple with ineffective functioning of malate shuttle which in turn depresses gluconeogenesis resulting in hypoglycemia.

2. The low NAD+ / NADH ratio inhibits the key enzymes of gluconeogenic pathway. This adds to profound hypoglycemia, particularly in alcoholics with underlying carbohydrate malnutrition.

3. Fatty acid synthesis is favored due to redox shift. This is accompanied by the inhibition of β-oxidation, resulting in esterification and storage of triglycerides in the liver. This results in hepatic steatosis. Increased synthesis of cholesterol, triacylglycerols and their accumulation in liver leads to fatty liver.

4. Increased lactate/pyruvate ratio due to reduced NAD+ / NADH ratio, decreases kidneys capacity to excrete uric acid. Thus uric acid concentration in plasma is increased, favours its deposition, leading to acute attack of gout.

5. Acetaldehyde forms protein adducts. This results in enzyme inactivation and decreased DNA repair. Thereby reduces rate of protein biosynthesis.

6. Many reactive oxygen species like superoxide ion radical (O2-), hydroxyl radicals (OH-) are produced during alcohol metabolism via MEOS. They interact with PUFA, cellular protein and DNA resulting in peroxidation leading to cell damage or death.

7. Mobilization of iron from ferritin occurs due to reduced NAD+ / NADH ratio, which intern interacts with H2O2 to produce hydroxyl and super oxide radicals which cause damage to liver cells.

8. Acetaldehyde is highly toxic may lead to direct hepatocellular injury and necrosis. It reacts with the sulphydryl groups of various enzymes, reducing their activity. It has effects on the protein-transport processes in hepatocytes such as glycoprotein secretion and receptor-mediated endocytosis. It alters antigenic characters of surface membranes, causes cell damage releasing cellular proteins into blood. All these effects cause formation of auto antibodies against membrane antigens, including cell membrane antigen and P4502E1.

9. In the mitochondria, most of the NADH is produced by ALDH. Through both of these pathways, alcohol oxidation vastly increases the availability of NADH to the electron transport chain in the mitochondria.

10. Cytokine interleukin-8 may have presumptive role in chronic alcoholic liver disease along with interleukin-1, interleukin-6, tumor necrosis factor-α. They cause liver damage by recruiting neutrophils to the hepatic parenchyma and promoting the release of proteolytic enzymes and superoxide ion.

11. The hepatocellular injury in alcoholics leads to the deposition of excess connective tissue in liver. This may be the possible mechanism for fibrosis. Acetaldehyde and lipid aldehydes promote collagen synthesis by lipocytes.

12. The phagocytic form of NAPDH oxidase expressed in Kupffer cells activated by several stimuli (i.e. alcohol metabolites and tumor necrosis factor-α) to produce ROS. Kupffer cells derived ROS consequently drive pro inflammatory effects and sensitize hepatocytes to undergo apoptosis, being involved in fibrogenesis and carcinogenesis [39].

Alcohol Metabolites and Adduct Formation

Alcohol metabolism by ADH and CYP2E1 produce reactive molecules, such as acetaldehyde and ROS, that can interact with protein building blocks (i.e., amino acids) and other molecules in the cell to form both stable and unstable adducts shown in Table 1.

Table 1: Different types of adducts formed during alcohol metabolism

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>METABOLITES AND ADDUCTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol metabolism</td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td>Non-enzymatic lipid peroxidation of unsaturated fatty acids</td>
<td>Malondialdehyde (MDA)</td>
</tr>
<tr>
<td>Lipid peroxidation of long-chain polyunsaturated fatty acids</td>
<td>4-hydroxynonenal (HNE)</td>
</tr>
<tr>
<td>Hybrid adducts with malondialdehyde and acetaldehyde</td>
<td>Malondialdehyde-Acetaldehyde Adduct (MAA)</td>
</tr>
<tr>
<td>Alcohol oxidation in the presence of iron</td>
<td>Hydroxyethyl radical (HER)</td>
</tr>
</tbody>
</table>

Acetaldehyde, the first metabolite of alcohol, and the aldehydic products of lipid peroxidation that are generated during alcohol-induced oxidative stress can bind to proteins and cellular constituent to form stable adducts [40]. Acetaldehyde can bind to reactive amino acid residues in several target proteins [41, 42]. However, not all amino acids in all proteins are equally likely to interact with acetaldehyde, and certain proteins seem to be particularly susceptible to forming adducts with acetaldehyde. These include the following:
Proteins found in the membranes surrounding the red blood cells (i.e., erythrocytes).
- Lipoproteins that consist of a protein and a fat component and which are associated with the risk of heart disease.
- Tubulin, a protein found in cell structures called microtubules that are essential for cell division and protein transport within cells.
- Hemoglobin, which is crucial for oxygen transport by the erythrocytes.
- Albumin, which is a protein found in the blood.
- Collagen, the major protein in connective tissue.
- Cytochrome enzymes, such as CYP2E1, which play a role in the metabolism of alcohol and many other substances.

Finally, acetaldehyde can form adducts by interacting with compounds known as biogenic amines, which include, among others, neurotransmitters such as serotonin and dopamine. (Biogenic amines are organic compounds formed during biochemical processes in plants and animals that carry a nitrogen atom as a central molecule.) These adducts may have pharmacological effects on the nervous system. Other protein adducts in alcoholics Aldehydic lipid peroxidation products also form Schiff base adducts with proteins in patients with excessive alcohol consumption [39]. A number of species have been identified, including adducts with malondialdehyde (MDA) [43], 4- hydroxynonalen (HNE) [44, 45], MDA-acetaldehyde adducts (MAA) and hydroxyethyl radicals (HER) [46].

MDA is a reactive dialdehyde originating from the non-enzymatic lipid peroxidation of a variety of unsaturated fatty acids, from lipid peroxidation that occurs during phagocytosis by monocytes and from arachidonic acid catabolism in thrombocytes [47]. HNE, produced by the free-radical-mediated oxidation of long-chain polyunsaturated fatty acids, can react with the sulfhydryl groups of proteins through a mechanism of the Michael addition type [48]. MDA adducts have been found in the liver of alcohol consumers [47] and hybrid MAA in the liver of alcohol-fed rats [49]. HER can also form adducts with hepatic proteins [50].

Possible functional effects of Adduct generation in tissues

Adduct formation may have many adverse functional consequences in physiological processes, such as interference with protein function, stimulation of fibrogenesis and induction of immune responses [45]. Consequently, tissue function and structure may be altered in a variety of ways. Acetaldehyde favours protein retention, with associated swelling of hepatocytes. Various mitochondrial functions are altered, particularly after chronic alcohol consumption, which sensitizes the mitochondria to the toxic effects of acetaldehyde [51]. Acetaldehyde stimulates collagen production in cultured myofibroblasts, and acetaldehyde–protein adducts also stimulate the production of antibodies directed against the acetaldehyde-derived epitopes [52, 53].

Adduct formation interferes with protein function particularly when there is a lysine residue in a functionally critical location, such as in tubulin and in lysine dependent enzymes [54]. Altered microtubule function may impair protein secretion and plasma membrane assembly, and the generation of reactive aldehydes may also contribute to alcohol-induced impairment of receptor-mediated endocytosis [55]. Acetaldehyde binding with cellular constituents can also stimulate fibrogenesis [56] and activate carcinogenesis. Alcoholics who have higher acetaldehyde levels due to polymorphisms and/or mutations in the genes coding for the enzymes responsible for acetaldehyde generation or detoxification have an increased risk of cancer [57]. Not all alcohol abusers develop liver fibrosis even with high alcohol consumption. On the other hand, the appearance of early fibrosis in zone 3, adjacent to the hepatocytes that are the site of protein-adduct deposition in the early phase of ALD, predicts the subsequent development of irreversible cirrhosis.

It has also been demonstrated that hepatic stellate cells (Ito cells) become readily activated under conditions involving acetaldehyde generation, enhanced oxidative stress and lipid peroxidation [58]. The triggering of immune mechanisms may represent one of the main pathways by which adduct formation contributes to the progression of ALD [45]. Aldehyde–protein adducts and hydroxyl radicals stimulate immunological responses directed against the specific modifications of proteins [43, 46].

Chronic alcohol administration to experimental animals has been shown to lead to the generation of circulating immunoglobulins with acetaldehyde adduct (neo antigen), anti-MDA-adduct (neo antigen) or anti-MAA adduct (neo antigen) specificity. Similar immunoglobulins and auto-antibodies recognizing CYP2E1-HER adducts have also been found in the blood of alcoholics. Both IgA and IgG responses have been reported [46, 52]. Enhanced production of cytokines by leukocytes is also associated with alcohol abuse, suggesting that excessive alcohol intake may also affect the regulation of immune responses [58]. Cytokine mediated cell-cell interactions may be important factors in the onset of alcohol induced liver damage, including the stimulation of fibrogenesis and inflammatory changes, although the mechanisms are poorly characterized. It has recently been suggested that the production of cytokines in the liver of alcoholic patients may be induced by increased level of endotoxin [43].

Pathogenesis of Alcohol Liver Disease: the mechanisms of liver damage

Pathogenetic process starts with the damage of cell membranes and cell organelles (especially mitochondria). Chronic alcohol consumption depresses the activity of all mitochondrial complexes, except complex II, [59, 60] several abnormalities in mitochondrial respiratory chain have been described in experimental models of chronic alcohol intoxication. These include: decreased activity and haeme content of cytochrome oxidase, [61] impaired electron transport and proton translocation through complex I, decreased cytochrome b content in complex III [62] and reduced function in ATP synthase complex [62]. As a result, the energy metabolism of liver cells can be severely impaired and this would lead to tissue damage.

Energy metabolism can also be altered by hypoxia. Chronic alcohol administration definitely enhances the oxygen uptake rate by liver cells because of the need of its metabolism, which mainly occurs in the centrilobular area of the liver lobule [63]. In
such circumstances, the liver blood flow increases, but such an increase does not match the requirements deriving from exalted alcohol metabolism [64]. Thus, centrilobular hypoxia ensues, which can be responsible for liver injury [65]. Centrilobular hypoxia can be further enhanced by the alcohol-induced changes in liver blood flow. In fact, alcohol infusion in a model of rat liver perfusion exerts a dose-dependent increase in portal pressure secondary to intra hepatic vasocostriction [66]. Thus, at high alcohol blood level, hypoxia might ensue from the combination of reduced perfusion and increased oxygen demand. When blood alcohol levels subsequently decline, lobular perfusion is restored and this can lead to reperfusion injury [67].

Liver damage occurs through several interrelated pathways. Alcohol dehydrogenase and acetaldehyde dehydrogenase cause the reduction of nicotinamide adenine dinucleotide (NAD) to NADH (reduced form of NAD). The altered ratio of NAD/NADH promotes fatty liver through the inhibition of gluconeogenesis and fatty acid oxidation. CYP 2E1, which is upregulated in chronic alcohol use, generates free radicals through the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP [67].

Chronic alcohol exposure also activates hepatic macrophages, which then produce tumor necrosis factor-alpha (TNF-alpha)[76]. TNF-alpha induces mitochondria to increase the production of reactive oxygen species. This oxidative stress promotes hepatocytes necrosis and apoptosis, which is exaggerated in the alcoholic who is deficient in antioxidants such as glutathione and vitamin E. Free radicals initiate lipid peroxidation, which causes inflammation and fibrosis. Inflammation is also incited by acetaldehyde that, when bound covalently to cellular proteins, forms adducts that are antigenic [68].

**Oxidative Stress (OS)**

Oxidative stress may be defined as a measure of the steady state level of the reactive oxygen or oxygen radicals in a biological system. Oxidative stress is actually "a disturbance in the prooxidant-antioxidant balance in favor of the former" [69]. Under this definition, pro-oxidants (electron-acceptors) damage proteins, DNA, and lipids when antioxidants (electron-donors) are insufficient to neutralize them. Although this definition is adequate when considering the direct damage caused to cellular components by reactive oxygen species, alterations in redox signaling may occur when the imbalance is not cytotoxic. In response to the increasing data about redox signaling, a new definition for oxidative stress was proposed as “an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage” and leads to cause many health problems like impairment of learning and memory [70].

**Free Radicals**

Free radicals are defined as molecules or molecular fragments with an unpaired electron. An unpaired electron being one that exists alone in an orbit. The electron imbalance makes the radical unstable and highly reactive as compared to biological molecules that have a paired electron. Free radicals may either be positively, negatively charged or electrically neutral in their characteristic. They are represented by a superscript dot (R•) [71]. The toxicity is due to a number of free radicals which are reactive oxygen and nitrogen species (ROS/RNS) produced during these normal cellular redox reactions as listed in Table 2.

**Table 2: Various type of free radicals (examples of ROS/ RNS)**

<table>
<thead>
<tr>
<th>Reactive Oxygen Species (ROSs)</th>
<th>Non-radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen radicals</td>
<td></td>
</tr>
<tr>
<td>O₂⁻ Superoxide Anion</td>
<td>ONOO⁻ Peroxynitrite</td>
</tr>
<tr>
<td>OH⁻ Hydroxyl radical</td>
<td>H₂O₂ Hydrogen peroxide</td>
</tr>
<tr>
<td>R⁻ Alkyl radical</td>
<td></td>
</tr>
<tr>
<td>RO⁻ Alkoxyl radical</td>
<td></td>
</tr>
<tr>
<td>ROO⁻ Peroxy radical</td>
<td></td>
</tr>
<tr>
<td>HQ⁻ Semiquinone radical</td>
<td></td>
</tr>
<tr>
<td>Reactive Nitrogen Species (RNSs)</td>
<td>Non-radicals</td>
</tr>
<tr>
<td>Nitrogen radicals</td>
<td></td>
</tr>
<tr>
<td>NO⁻ Nitric oxide radical</td>
<td>ONOO⁻ Peroxynitrite</td>
</tr>
<tr>
<td>NO₂⁻ Nitrogen dioxide radical</td>
<td></td>
</tr>
</tbody>
</table>

Endogenous sources of ROS are, the mitochondrial electron transport chain, respiratory burst by phagocytes, beta oxidation of fatty acids in peroxisomes, auto-oxidation of amino acids, reperfusion ischemia injury, xanthine oxidase and cyclooxygenase and lipoxygenase reactions in eicosanoid biosynthesis. Transition metals such as iron, copper and molybdenum play a key role in the initiation of chain reactions of oxidation [72]. ROS are generated by phagocytic cells as cytotoxic agents to fight invading microorganisms, a process known as the respiratory burst. NADPH oxidase, a membrane bound enzyme catalyses the reduction of oxygen to superoxide anion. The ROS are also produced by electron leakage from the electron transport chain in mitochondria where molecular oxygen is sequentially reduced to superoxide and hydrogen peroxide. Another major source of ROS in tissues is the xanthine oxidase which catalyses the oxidation of hypoxanthine to xanthine and then xanthine to uric acid, reducing oxygen by one or two electrons resulting in the formation of superoxide or hydrogen peroxide. This enzyme is a flavoprotein containing transitional metals molybdenum and iron [73].
In normal physiological conditions, ROS are produced in a controlled manner at low concentrations and function as signaling molecules regulating vascular contraction-relaxation and cell growth. Increased ROS bioavailability and altered redox signaling (oxidative stress) have been implicated in chronic diseases including hypertension[74]. External sources of ROS include, environment (e.g. sun exposure, X-rays), toxins (e.g. food toxins, drugs), ozone, automobile exhaust, and lifestyle stressors such as cigarette smoking and, excessive alcohol consumption as shown in Figure 3.

Figure 3: Reactive Oxygen Species (ROS) generated by endogenous as well as exogenous sources.

Nitric oxide (NO) is the major reactive nitrogen species synthesized from L-arginine by the enzyme nitric oxide synthase. Peroxynitrite (ONOO-) is another powerful RNS [72, 73]. Some of these are free radicals that can damage many components of cells, including lipids, proteins, and nucleic acids [75, 76]. Physiological levels of ROS are beneficial for cells. ROS can regulate transcription by activating the transcription of specific genes and can act in the immune system as effect or molecules against pathogens. Many components of the cell, including mitochondria, endoplasmic reticulum, peroxisomes, membranes, and cytosol, can be sources of ROS.

In general, there is a balance between the production of ROS and cellular antioxidant agents. The accumulation of low to moderate levels of ROS is generally counterbalanced by the cell’s endogenous antioxidant defense system. Antioxidant agents act jointly to remove various ROS produced by free radical reactions. Indeed, antioxidant activity may be a consequence of ROS production. If the amount of ROS increases, and if these products destroy the apparatus by which antioxidant agents are produced, the cellular defense system is eventually incapacitated. It appears that higher levels of ROS induce necrotic cell death whereas lower levels lead to apoptosis.

Oxidative Stress and Alcohol Liver Disease (ALD)

Oxidative stress plays a pivotal role in the development of ALD as in Figure 4 [77, 78]. Acetaldehyde/Alcohol leads to increased liver oxidative stress via generation of highly reactive oxygen species (ROS) and adducts. ADH generates acetaldehyde, which is subsequently oxidized to acetate by ALDH. Acetaldehyde can form hybrid-adducts with reactive residues (e.g.
malondialdehyde adduct) acting on proteins or small molecules (e.g. cysteines), mediating lipid peroxidation and nucleic acid oxidation [78].

![Diagram of Pathogenesis of hepatic and metabolic abnormalities after alcohol ingestion due to oxidative stress](image)

**Figure 4:** Pathogenesis of hepatic and metabolic abnormalities after alcohol ingestion due to oxidative stress

MEOS: Microsomal Alcohol Oxidation System and ADH: Alcohol Dehydrogenase.

Further oxidations in alcohol metabolism are accompanied by an excessive reduction of nicotinamide adenine dinucleotide (NAD), with a shift in the NADH/NAD ratio. Under normal circumstances, reduction of NAD (NAD: NADH) is finely regulated by the cell Krebs cycle. The shift caused by excessive alcohol consumption is thought to impair carbohydrate and lipid metabolism, finally causing impairment of gluconeogenesis and diversion of metabolism to ketogenesis and fatty acid synthesis [77, 78].

The increased amount of reducing equivalents, such as NADH, leads to their shuttering into mitochondria, which induces the electron transport chain components to assume a reduced state. This facilitates the transfer of an electron to molecular oxygen to generate reactive species as superoxide anion [79]. Mitochondrial ROS generation can also derive from the alterations produced in mitochondrial complexes I and III, which have been discussed above. In fact, such alteration can also promote superoxide anion generation within the mitochondria. Thus, mitochondria represent a main site where huge amount of ROS are generated, leading, in turn, to cell damage and necrosis.
Finally, the NADH-induced inhibition of mitochondrial b-oxidation leads to accumulation of intracellular lipids, thus promoting steatosis [77, 80]. Excessive alcohol consumption is also associated with the enzymatic induction of CYP2E1 pathway of alcohol metabolism. The recruitment of this pathway may indirectly contribute to ALD development by excess production of superoxide radicals via the interaction of CYP2E1 with cytochrome reductase, which leads to electron leaks in the respiratory chain and ROS production [79]. The species produced in this cascade can interact with iron (Fenton reaction) generating even more potent hydroxyl, ferryl and perferryl radicals which perpetuate liver damage [81].

Indeed, liver inflammation is initiated by Kupffer cells, and it involves the formation and release of many inflammatory mediators, which include neutrophils, lymphocytes, and other inflammatory cells to damaged regions [82]. Hepatocytes and infiltrating inflammatory cells interact in a complex and multifactorial process through soluble mediators, surface receptors, and adhesion molecules [83]. In response to hepatocellular stress, pro-inflammatory cytokines, e.g., interleukin-1, interleukin-6, and tumor necrosis factor (TNF)-α are released. These agents stimulate neighboring hepatocytes and other non-parenchymal cells [84]. Proinflammatory cytokines such as TNF-α can induce the formation of ROS in hepatocytes, [85] where they can play a role in a large variety of activities, including Ca2+ accumulation, circulatory and transport function, NO synthesis and metabolism, cytokine gene expression, caspase activity, growth factor synthesis and activity, DNA fragmentation, and Na+ influx [86]. ROS can react with fatty acid chains of membrane phospholipids. Reaction products are mostly seen on the outer membrane of the mitochondria, suggesting that lipid peroxidation occurs mainly at the mitochondrial membrane and supporting the finding of considerable mitochondrial changes in hepatocytes in alcoholic hepatitis, non-alcoholic steato-hepatitis, [87] and hepatitis C [88]. Some of the products of lipid peroxidation can lead to loss of integrity, resulting in necrosis. It is suggested that to selectively kill hepatocytes by lipid peroxidation, a combination of oxidant stress, iron mobilization, and depletion of cellular antioxidants is necessary [89]. Excessively high levels of iron are stored in the hepatocytes of patients with non-alcoholic steato-hepatitis, alcoholic hepatitis, or hepatitis C.

The over-accumulation of iron causes oxidative stress in the hepatocytes [90]. It is a fact that certain lipid peroxidation products are potent chemo tactic factors for neutrophils and can modulate reactive oxygen formation [91]. Hepatocytes, with their more potent antioxidant systems, are less susceptible to oxidant stress than non-parenchymal cells [92]. Kupffer cells and endothelial cells are more exposed or sensitive to oxidative stress-related molecules. Under inflammatory conditions, in which activated complement factors stimulate Kupffer cells to produce reactive oxygen in the hepatic vasculature, complement induces the enhanced sinusoidal release of GSH from hepatocytes [93]. Hepatocytes contain about 10% of the total body pool of GSH [94], GSH in the space of Disse non-enzymatically reacts with hydrogen peroxide, peroxynitrite, and hypochlorous acid [94]. It also affects the transcription of proinflammatory/anti-inflammatory cytokine genes, liver regeneration through cytokine-mediated nuclear factor κ binding (NF-κB) induction, NO bioavailability, and energy metabolism [95]. Depletion of GSH may be both a reason for and a consequence of liver damage. GSH levels in the liver and circulation have been reported to be decreased in patients with alcoholic and viral cirrhosis and HCV-related chronic hepatitis [96]. Enhanced production of ROS and the altered GSH pool contribute to programmed death by activating gene expression for transcription factors such as NF-κB, leading to up-regulation of proinflammatory cytokines, chemokines, adhesion molecules, Fas ligands, survival genes, etc., which consecutively activate the cascade of caspases (apoptosis) or induce the release of cytochrome c and the depletion of ATP at the mitochondrial level (necrosis) [97]. Necrotic cell death is linked to the opening of the permeability transition pore and the onset of mitochondrial membrane permeability transition (MPT), [98] which leads to mitochondrial uncoupling and loss of membrane potential.

A significant magnitude of oxidant stress causes oxidation of mitochondrial NAD(P)H and reactive oxygen formation by mitochondria, both of which increase mitochondrial free Ca2+ levels [99]. The MPT is induced by an increase of mitochondrial Ca2+ directly or through the activation of mitochondrial serine proteases (calpains). In addition, cytosolic calpains promote membrane blebbing via degradation of cytoskeleton proteins [100]. The combination of these events leads to rapid necrotic cell death. The induction of the cytochrome P450 enzyme system, the endotoxin-induced cytokine expression in Kupffer cells, and neutrophil infiltration further enhance the production of ROS and deplete ATP reserves in hepatocytes. ROS generated from neutrophils migrate into the hepatocytes and potentiates the shift of apoptosis to necrosis. Another contribution to cell death derives from the adhesion of cytotoxic lymphocytes that release proteases and perforin from cytotoxic granules, particularly in presence of ROS, which mediate the lymphocyte-Fas/Fas ligand interaction [101].

Endotoxin is another important element in the pathogenesis of alcohol-induced liver damage. Circulating endotoxin levels increase after alcohol intake and endotoxins may trigger both cytokine release and oxidative stress. Fibrogenesis within the liver is a consequence of the activation of collagen-producing stellate cells. The pathway leading to the activation of stellate cells is not certain, and may include a number of simultaneous processes, such as direct injury from reactive oxygen species or through intermediate steps which include the expression of interleukins (IL), such as tumor necrosis factor (TNF), IL1, IL6, IL8 and the transforming growth factors [102]. The profile of cytokines released in response to alcohol, lipid peroxidation, circulating endotoxin and Kupffer cell activation is influenced by genetic polymorphisms [103]. These observations are of potential importance in the discovery of therapies for alcoholic liver disease by antioxidant agents, or by blocking the release of cytokines and growth factors precipitating collagen deposition. In conclusion, the role of alcohol to promote oxidative stress in ALD due to free radicals is well known. However, additional studies are needed to further clarify the role of ROS in alcohol induced liver injury which helps in providing the information to set strategies to prevent or attenuate the toxic effects of alcohol.

The mechanisms of cellular injury due to oxidative stress are complex. Potential mechanisms include chemical modification of biological molecules and further stimulation of the host immune response, including the release of cytokines, with resultant
inflammatory and fibrotic responses, direct activation of stellate cells and, finally, inhibition of the synthesis of S-adenosyl methionine.

**Defence system against oxidative damage**

As mentioned above, in order to minimize the generation and counterbalance the damaging effects of reactive species, eukaryotes have developed a comprehensive defence system. The defence system comprises of antioxidants which works at different molecular aspects.

Antioxidants are molecules or compounds that act as free radical scavengers. Most antioxidants are electron donors and react with the free radicals to form innocuous end products such as water. These antioxidants bind and inactivate the free radicals. Thus, antioxidants protect against oxidative stress and prevent damage to cells. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general antioxidant systems either prevent these reactive species from being formed or remove them before they can damage vital components of the cell. Antioxidants may be synthesized in the body or obtained from the diet.

Preventive antioxidants suppress the formation and decrease the reactivity of reactive species. They are classified as enzymatic and non enzymatic as shown in Figure 5. The enzymes involved in this process include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, peroxiredoxins and thioredoxin reductase. The non enzymatic components include polypeptides like thioredoxins, glutaredoxins and sulfiredoxins; metal binding proteins like transferrin, albumin; low molecular weight antioxidants like glutathione, uric acid; dietary antioxidants like vitamin E, ascorbic acid, carotenoids and polyphenols.

---

**Figure 5: Classification of Antioxidants**

Another aspect in this defence is the repairing process and includes repair enzymes, which repair the damage and reconstitute membranes and DNA, for example lipase, DNA repair enzymes and transferases. The defence system mentioned above could be called as “antioxidant defence system” since an antioxidant defined by Halliwell is any substance that delays, prevents or removes oxidative damage to a target molecule [104].

**Antioxidant Enzymes**

The protection of cells against damage from oxygen and its metabolites can be accomplished through enzymatic and non-enzymatic means. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are considered to be the primary
antioxidant enzymes, since they are involved in the direct elimination of reactive oxygen species. Glutathione-S-transferase (GST), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) are secondary antioxidant enzymes which help in the detoxification of reactive oxygen species by decreasing peroxide levels by GST or by maintaining a steady supply of metabolic intermediates like glutathione as by GR and NADPH by, G6PD for the primary antioxidant enzymes.

Superoxide dismutase (SOD) is a very important enzyme that functions as a cellular anti-oxidant. It is present as isoenzymes in different organelles as copper-zinc SOD in cytoplasm, as manganese SOD in mitochondria in order to maintain a low concentration of superoxide anion. There is also an extracellular form of superoxide dismutase in plasma, lymph and synovial fluid that is different from the intracellular forms of the enzyme. The extracellular enzyme may function at cell surfaces. SOD catalyzes the dismutation of superoxide anion and the absence of this enzyme is lethal. The amount of superoxide dismutase is controlled by specific redox-sensitive genes in cells [105].

Catalase, a heme containing protein that catalyzes the reaction in which hydrogen peroxide is detoxified. Catalase is a cytoplasmic enzyme, usually found in peroxisomes of the cells and is expressed in all types of cells except erythrocytes as they do not contain the peroxisomes. Catalase provides a protective role that is similar to that of glutathione peroxidase because both are important means of removing hydrogen peroxide [106]. Both catalase and glutathione peroxidase are important in hydrogen peroxide detoxification.

Glutathione peroxidase is a cytoplasmic and mitochondrial enzyme, important for detoxifying $\text{H}_2\text{O}_2$ in almost all the cells. It is a seleno protein, which contains a seleno-cysteine amino acid at the active site instead of a normal cysteine [107]. The selenium that replaces the normal sulfur in this amino acid has enhanced nucleophilic properties and ionizes more readily to release a proton. It is a much more effective catalyst in the reaction catalyzed by this enzyme.

The flavoprotein, glutathione reductase uses the reducing power for the pentose phosphate pathway (NADPH) to keep the glutathione pool in cell in a highly reduced state. Even when large amounts of hydrogen peroxide are present this enzyme is very effective at reducing the cellular glutathione pool. The net result of this cycle is to use NADPH to reduce hydrogen peroxide to water, a process that requires two electrons [108]. Other reductases can also catalyze reactions that reduce lipid peroxides, instead of hydrogen peroxide.

Glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme of the pentose phosphate pathway, has long been regarded as important in the biosynthesis of the sugar moiety of nucleic acids [109]. Until recently, the role of this housekeeping enzyme in the cell response to oxidative stress was limited to human erythrocytes that lack any other NADPH-producing route [110, 111]. However, recent results have demonstrated that this enzyme also plays a protective role against ROSs in nucleated eucaryotic cells that possess alternative routes for the production of NADPH.

Glutathione-S-transferase (GST) is glutathione dependent antioxidant enzymes, catalyzes the reaction between the-SH group and potential alkylating agents, rendering them more water soluble, suitable for transport out of the cell [112]. It shows high activity with lipid peroxides, [113] particularly high levels in the liver and serves in detoxification metabolism [114].

**Non-Enzymatic Antioxidants**

The non-enzymatic antioxidants are again classified into hydrophilic and hydrophobic. Hydrophilic antioxidants can dissolve into blood and cytosol and react with free radicals. Hydrophobic antioxidants protect the cell membrane from lipid peroxidation, a mechanism by which free radicals degrade the membrane lipids [115]. The role of antioxidants in scavenging the deleterious effects of free radicals is complex, which depend on the interactions of various metabolites and enzyme systems having synergistic and interdependent effects on one another [116]. The performance level of antioxidants also depends on the concentration, reactive potentiality with the specific free radical, interaction and function with other antioxidant family members [117].

β- Carotene, also known as pro-vitamin A, having incredible antioxidant power by which it effectively protects the cells against multiple types of cancer, especially lung cancer. A part from this, β-carotene is particularly helpful against free radical damage and improves immune response by increasing the T-helper cells [118]. β-carotene alone wipes out free radicals formed in the body especially singlet oxygen. With the help of Vitamin E, β-carotene completely eliminates the oxygen species. Butternut squash, turnip greens, kale, beet greens, red peppers, tomatoes, collard greens, apricots, cantaloupe, peaches, prunes and sweet potato are the richest sources of β - carotene.

Pyridoxine (vitamin B6) is a significant vitamin, which reduces the toxicity of the homocysteine by converting it to glutathione. The importance of this antioxidant is best exemplified in the depriving rats of dietary vitamin B6 severely compromises their antioxidant defenses, making them more sensitive to oxidative stress [119]. Supplementation with vitamin B6 reduced the oxidative stress levels by abnormally elevating the homocysteine levels in homocysteinemic rats as a consequence of depriving animals of folic acid [120]. The combination of creatine and pyridoxine will enhance the antioxidant status, hastens muscle recovery and accentuate muscle anabolism in the humans [121]. Pyridoxine represents yet another important possibility to reduce the harmful effects of oxidative stress. Thiamine (Vitamin B1) is getting prominence as a promising antioxidant in plants especially under abiotic
stress conditions. In humans the deficiency of thiamine causes muscle cramps and Beriberi disease, which is due to the rapid activity of the free radicals in muscle cells. However, it is yet to be proved the exact role of thiamine in scavenging the toxicity of free radicals in humans.

Ascorbic acid (Vitamin C) is the most potent antioxidant, which promotes the elimination of free radicals generated by the human body as well as the external sources. The action of free radicals on skin cells is one of the most important factors in skin aging. Being water soluble ascorbic acid can work both inside and outside the cells to combat free radical damages by donating the electrons to free radicals and neutralizing their reactivity [122]. Ascorbic acid also protects liver from free radical damage and DNA from the attack of free radicals and mutagens which prevents harmful genetic alterations within the cells and protects lymphocytes from mutations to the chromosomes [123]. Vitamin C is also an essential co-factor for many enzymes, and its depletion lowers levels of internally produced antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. Low levels of these antioxidants may be associated with increased rates of cancers in humans, whether or not they consume alcohol.

Interaction of ascorbic acid with folic acid (Vitamin B6) prevents free radical damage to the lipids reducing lipid peroxidation, blood pressure, cholesterol levels, help in thinning of blood and protects it against oxidation [124]. β-carotene and selenium are formed when ascorbate is added to α-tocopherol (vitamin E), helps in prevention of stroke[125] and in alleviation of pancreatitis, or an inflammation of the pancreas [126]. Regular consumption of ascorbic acid (1g/day) protects against Low Density Lipoprotein (LDL) cholesterol [127].

Vitamin E is the collective name of a group of fat soluble compounds with distinctive antioxidant activities [128]. Vitamin E naturally exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol with varying levels of biological activities among which alpha (α-tocopherol is the only recognized form of vitamin E with sufficient concentrations in blood compared with other forms [129]. Vitamin E controls the production of ROS during the oxidation of fats by neutralizing the free radicals thereby decreasing the intensity of the damage to the tissues and macromolecules. Vitamin E prevents or delays the chronic diseases including CALD, macular degeneration, osteoarthritis, and prostate enlargement which are associated with the free radicals [130]. α-tocopherol serves as an effective anti-inflammatory agent and protects the liver from damage [131].

Glutathione is an effective antioxidant and important component of antioxidant network that protects our body against the effects of free radicals. Glutathione is a small protein which is built with three amino acids cysteine, glutamic acid, glycine and generally exists in reduced (GSH) and oxidized states (GSSG) as shown in Figure 6. In reduced state, GSH can donate an electron to stabilize free radical. During this donation the GSH will become highly reactive and reacts with another GSH to form GSSG. GSSG will be reduced to GSH by the enzyme glutathione reductase [132]. The antioxidant property of glutathione includes detoxification from heavy metals, solvents, pesticides etc. and transforms them into a form which can be excreted along with urine. It is also involved in counteracting effects of free radicals in the body by oxidation.

![Glutathione Redox Cycle](image)

**Figure 6: Glutathione Redox Cycle CAT: Catalase, GSH-Px: Glutathione peroxidase, GSH- Oxidised Glutathione, GSSG-R- Glutathione Reductase, GSSG- Reduced Glutathione**

Coenzyme Q10 is a natural compound found in the mitochondria of the cell involved in the manufacture of ATP, a major source of cell's energy which drives a number of biological processes including muscle contraction and the synthesis of proteins [133]. Coenzyme Q performs two major roles (i) carrier of electrons from respiratory complexes I and II to complex III, and (ii) anti-oxidant quenching of free radicals [134]. Coenzyme Q10 protects the body against free radical invasion and has been used both as preventative and treatment aid for many organ disorders [135,136].

Alpha-lipoic acid (α-lipoic acid is synthesized by almost all types of tissues and capable of solubilising in both water and fats, hence can work throughout the body [137]. Alpha-lipoic acid, a dithiol compound derived from octanoic acid, which plays an essential role in mitochondrial dehydrogenase reactions. Alpha-lipoic acid acts by multiple mechanisms both physiologically and
pharmacologically. Pharmacologically improves glycemic control, polyneuropathy. Physiologically as an antioxidant, alpha-lipoic acid directly terminates free radicals, chelates metal ions, increases cytosolic glutathione and vitamin C [138]. This lowering of blood sugar levels, reducing pain, burning, itching, tingling, and numbness in people who have nerve damage caused by diabetes called peripheral neuropathy [139].

Lutein and lycopene belongs to the carotenoid family. Carotenoids are the powerful antioxidants that quench free radicals especially singlet oxygen, which is produced during exposure to ultraviolet light, which is the primary cause of skin aging [140]. The biological mechanisms for the protective effects of lycopene including modulation of functions and antioxidant properties are only partially known. Recent studies indicate that a regular intake of lutein is associated with a reduced risk of coronary heart disease and certain types of cancer [140, 141]. Lycopene is found in red fruits. Lycopene controls lung, stomach and prostate cancers by activating special cancer preventive enzymes such as phase II detoxification enzymes, which remove harmful carcinogens [142]. Presence of lycopene in liver and lung tissues protects lymphocytes from ROS damage [143].

Flavonoids are phenolic substances which have been isolated from wide range of vascular plants. These compounds are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. So far, 8,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages. Flavonoids play a wide variety of roles in protecting human health as antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, antioxidants, antimicrobials, photo-receptors, visual attractors, feeding repellants, and for light screening [144]. However, most interest has been devoted to the antioxidant activity of flavonoids, due to their ability to reduce free radical formation and to scavenge free radicals. The capacity of flavonoids to act as antioxidants in vitro has been the subject of several studies in the past years, and important structure-activity relationships of the antioxidant activity have been established [145].

Selenium is a non-metal which exists in multiple oxidation states (+2, +4, +6). Within biological systems this element is a constituent of the amino acids that compromise proteins and an essential component of the glutathione peroxidase enzyme system with a significant function of protecting the cell from the oxidative stress and free radical formation. Selenium can be considered the "rate-limiting" substrate in the GSH-GSSG oxido-reduction [146] whose deficiency will affect the synthesis of peroxidase enzyme affecting the antioxidant protection by severely reducing the GSH-GSSG levels.

Zinc is the central component of more than 1,000 proteins including DNA-binding proteins with zinc fingers, copper/zinc superoxide dismutase (Cu/Zn SOD) and several proteins involved in DNA-damage repair such as p53, which is mutated in half of human tumors [147]. Insufficient zinc intake can impair antioxidant defences and compromise DNA-repair mechanisms, making the cell highly susceptible to oxidative DNA damage. Thus, deficits in zinc intake could have a significant impact on the development of cancer [148].

**CONCLUSION**

Long-term intake of more than 30g of absolute alcohol per day increases the risk of ALD; liver disease is nearly certain in long-term consumption in excess of 80 g of absolute alcohol per day. Alcoholic liver disease may take the chronic form (steatosis, steato-hepatitis, fibrosis, cirrhosis) or that of acute hepatitis due to oxidative stress under the influence of free radicals. Enzymatic and non-enzymatic antioxidants counteract the toxic effects of free radicals in human body, which is being supplemented in physiological, biochemical mechanisms. An appropriate balance of enzymatic and non-enzymatic antioxidant defense is necessary for withstanding the destruction caused by the active oxygen species and maintaining the human health. Such studies will open new doors in pharmacy and drug designing studies to identify new drug targets for successful amelioration of free radicals.

Hence, the work carried out this study entails that markers of oxidative stress and other enzymatic and non-enzymatic antioxidants status can reflect the course of disease and also the effectiveness of the drugs regimen. The investigation definitely will help to comprehend the role of oxidative stress in pathogenesis of ALD. Keeping in view the rising incidence of ROS, it will have paths towards better management and treatment of the disease. This is also provides a theoretical basis for the development of novel therapeutic strategies, such as antioxidant supplementation. The treatment with antioxidants in the initial stages of diseases such as ALD, may be useful as secondary/adjunctive therapy besides conventional drugs to prevent further oxidative damage.

**Authors’ Statements**

**Competing Interests**

The authors declare no conflict of interest.

**REFERENCES**


