***Review Article***

# Free radicals: Health implications and their mitigation by herbals


## Abstract

Free radicals pose a serious threat to tissues and vital organs, especially membrane lipids, proteins and nucleic acids of cells. Overproduction of reactive oxygen/ nitrogen species (ROS/RNS) and other related radicals lead to oxidative stress which has been implicated in aging and a number of diseases. Free radicals react with biomolecules and cause lipid peroxidation, loss of enzyme activity, mutation and carcinogenesis. A number of degenerative diseases including cardiovascular disease, diabetes, and adverse hepatic conditions have been attributed to accumulation of free radicals. Diseases resulting from radical overload might also lead to different types of cancers. However free radicals at low or moderate levels are vital to human health. ROS and RNS produced in a well regulated manner help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. Cellular antioxidant enzyme systems including superoxide dismutase, catalase, glutathione peroxidases/reductase, peroxiredoxins along with non enzymatic antioxidants viz., tocopherols, vitamin C, and glutathione etc., apart from several dietary components protect cells and organisms from the lethal effects of excessive ROS production. Natural products of plant origin have been used in traditional medicine for the treatment of diseases resulting from radical overload. The diversity of phytochemicals such as polyphenols, flavonoids, carotenes and saponins etc. present in plants and dietary components provide drug leads for the development of novel therapeutic agents. This review deals with the components of free radical biology, their adverse consequences in humans and amelioration of diseases by botanical therapeutics.



**Key words:** Reactive oxygen species, Oxidative stress, Cancer, Aging, Diabetes, Plant Products.


## 1. INTRODUCTION

Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules in healthy human cells and cause protein and DNA damage along with lipid per-oxidation. It may be defined as an imbalance between free radicals and antioxidants in our body (Figure 1). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [1]. In general, free radicals are very short lived, with half lives in milli, micro or nanoseconds. The most common reactive oxygen species (ROS) include superoxide ($O^{2-}$) anion, hydrogen peroxide ($H_2O_2$), peroxyl ($ROO^{-}$) radicals, and reactive hydroxyl ($OH^{\bullet}$) radicals. The nitrogen derived free radicals are nitric oxide ($NO^{\bullet}$) and peroxynitrite anion ($ONOO^{-}$). Under physiological conditions, ROS formation and elimination are delicately balanced. However, enhanced activity of oxidant enzymes and/or reduced activity of antioxidant enzymes lead to oxidative stress. Majority of the diseases/disorders are mainly linked to oxidative stress produced due to free radicals [2, 3].

ROS have been implicated in over a hundreds of disease states which range from arthritis, connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome [4, 5]. Pathological conditions that predispose to cardiovascular events, such as hypertension, hypercholesterolemia, and diabetes, are associated with oxidative stress. Antioxidant therapy has gained an immense importance in the treatment of these diseases. Antioxidants have been reported to prevent oxidative damage caused by free radicals and ROS, and may prevent the occurrence of diseases such as cancer and aging. They can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also acting as oxygen scavengers [6, 7, 8]. Many phytochemicals have been found to play as potential antioxidants. Present review summarizes the causes and consequences of free radical generation, antioxidants and use of plants derivatives in controlling diseases.

## 2. FREE RADICALS

Free radicals are atoms, molecules or ions with unpaired electrons that are highly unstable, short lived and active towards chemical reactions with other molecules. They may be derived from oxygen, nitrogen and sulfur [9, 10]. Internally, free radicals are produced as a normal part of metabolism within the mitochondria, through xanthine oxidase, peroxisomes, inflammation processes, phagocytosis, arachidonate pathways, ischemia, and physical exercise. External factors that help to promote the production of free radicals are smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents and ozone. It is paradox that these elements, essential to life (especially oxygen) have deleterious effects on the human body through these reactive species [9].

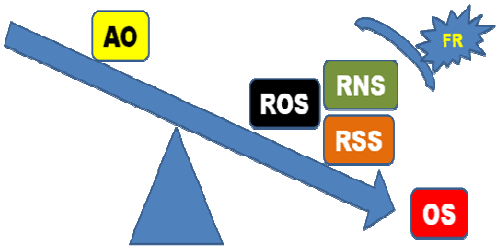



**Fig. 1.** Effect of imbalance between antioxidants and free radicals (Abbreviations: AO-antioxidant, ROS-reactive oxygen species, RNS-reactive nitrogen species, RSS-reactive sulphur species, FR-free radicals, OS-oxidative stress).

### 2.1 Reactive oxygen and nitrogen species (ROS and RNS)

Free radicals derived from oxygen and nitrogen are known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively. Formation of ROS and RNS in the cells can occur by enzymatic and/or non-enzymatic reactions. Enzymatic reactions include those involved in the respiratory chain, the prostaglandin synthesis, the phagocytosis, and the cytochrome P450 system [11]. Some of ROS molecules are extremely reactive, such as the hydroxyl radical, while some are less reactive (superoxide and hydrogen peroxide) [5, 12]. The superoxide anion created from molecular oxygen by the addition of an electron is, in spite of being a free radical, not highly reactive. It lacks the ability to penetrate lipid membranes and is therefore enclosed in the compartment where it was produced. The formation of superoxide takes place spontaneously, especially in the electron-rich aerobic environment in

vicinity of the inner mitochondrial membrane with the respiratory chain. Superoxide (as well as hydrogen peroxide) is also produced endogenously by flavoenzymes, e.g., xanthine oxidase activated in ischemia-reperfusion [13, 14]. Other superoxide-producing enzymes are lipoxygenase and cyclooxygenase [15, 16]. Hydrogen peroxide plays a radical forming role as an intermediate in the production of more reactive ROS molecules including hypochlorous acid by the action of myeloperoxidase, an enzyme present in the phagosomes of neutrophils [17]. Most importantly, hydrogen peroxide forms hydroxyl radical in a reaction catalyzed by metal ions ($Fe^{2+}$or $Cu^{+}$), often bound in complex with different proteins or other molecules by a reaction known as the Fenton reaction [18, 19].

Nitric oxide (NO) is formed from L-arginine by one of the three NO synthase (NOS) isoforms. The three isoforms are nNOS (identified constitutive in neuronal tissue), iNOS (inducible by cytokines in activated macrophages and liver) and eNOS (identified constitutive in vascular endothelial cells) [20]. NO is rapidly oxidized by oxyhemoglobin to form nitrate, the major end stable oxidation product of NO in the body. NO also reacts with glutathione to form nitrosothiol or with heme to yield heme-NO. Physiologically, nitrosothiol can serve as a vehicle to transport NO in plasma, thereby increasing the biological half-life of physiologic concentrations of NO [21, 22].

## 2.2 Physiological functions of free radicals

ROS and RNS are involved in many physiological activities and function as cellular signaling agents. Activation of phagocytes produces ROS in amounts enough to kill intruding bacteria [23]. In this system ROS are produced by the NADPH oxidase complex that converts O2 to O2•- [24, 25]. Superoxide is then reduced in the phagosome by SOD to $H_2O_2$ that can be further converted to HOCl by myeloperoxidase [26]. Hypochlorous acid may then spontaneously form hydroxyl radical. The two highly reactive ROS molecules thereby formed in phagosomes (HOCl and •OH) are highly toxic to bacteria ingested by the phagocyte and carry the direct antimicrobial effects of ROS. The hypochlorous acid produced in the myeloperoxidase reaction is also an important part of the antimicrobial defense by destruction of the DNA anchoring at the bacterial membrane, resulting in cessation of DNA replication [27].

ROS can directly affect the conformation and/or activities of all sulfhydryl-containing molecules, such as proteins or GSH, by oxidation of their thiol moiety. This type of redox regulation affects many proteins important in signal transduction and carcinogenesis such as protein kinase C, $Ca^{2+}$-ATPase, collagenase, and tyrosine kinases [28], among many other enzymes and membrane receptors [29]. For several transcription factors, ROS function as physiological mediators of transcription control. Well-known examples of redox-sensitive transcription factors are Nuclear Factor-κB (NF-κB) and Activator Protein-1 (AP-1) [30]. Activator Protein-1, a dimer of gene products from the *Jun* and *Fos* proto-oncogene families, expression is induced by several pro-oxidant conditions, including different types of irradiation [31, 32]. Nitric oxide (NO) is one of the most important signaling molecules. Physiologic levels of NO produced by endothelial cells are essential for regulating the relaxation and proliferation of vascular smooth muscle cells, platelet aggregation, leukocyte adhesion, angiogenesis, vascular tone, thrombosis, and hemodynamics. In addition, NO produced by neurons serves as a neurotransmitter, and NO generated by activated macrophages is an important mediator of the immune response [33, 34].

## 2.3 Molecular damage induced by free radicals

All the biological molecules present in our body are at risk of being attacked by ROS. It is estimated that every day a human cell is targeted by the hydroxyl radical and other such species on an average of 105 times inducing oxidative stress [33]. The main targets of ROS and other free radicals are proteins, DNA and RNA molecules, sugars and lipids [34-37]. Membrane lipids present in sub-cellular organelles are highly susceptible to free radical damage. During lipid per-oxidation a large number of toxic byproducts are also formed that can have effects at a site away from the area of generation, behaving as second messengers. The damage caused by lipid peroxidation is highly detrimental to the functioning of the cell [38]. Oxidation of proteins by ROS/RNS can generate a range of stable as well as reactive products such as protein hydroperoxides that can generate additional radicals particularly upon interaction with transition metal ions. Table 1 summarizes the mechanisms involved in free radical damage of biomolecules. Oxidative damage to DNA is a result of interaction of DNA with ROS or RNS. The C4-C5 double bond of pyrimidine is particularly sensitive to attack by hydroxyl radical, generating a spectrum of oxidative pyrimidine damage products, including thymine glycol, uracil glycol, urea residue, 5-hydroxydeoxyuridine, 5-hydroxydeoxycytidine, hydantoin and others. 8-Hydroxydeoxyguanidine (8-OHdG) has been implicated in carcinogenesis and is considered a reliable marker for oxidative DNA damage [38].

**Table 1.** Mechanisms involved in free radical mediated damage to biomolecules

| Targets of free radicals | Mode of damage |
|---|---|
| Proteins | Oxidative modification of a specific amino acid.<br>Free radical-mediated peptide cleavage.<br>Formation of protein cross -linkage due to reaction with lipid peroxidation products [9]. |
| DNA and RNA | Production of base-free sites.<br>Deletions, modification of bases.<br>Frame shifts.<br>Strand breaks.<br>DNA–protein crosslink and chromosomal arrangements.<br>Oxidation of DNA by hydroxyl radicals [39, 40]. |
| Sugars | Formation of oxygen free radicals during early glycation could contribute to glycoxidative damage [40].<br>Short sugar fermentation products (glycoaldehyde) due to autoxidation produce superoxide radical [40]. |
| Lipids | Lipid peroxidation takes place by the abstraction of hydrogen atom from a methylene carbon of fatty acid side chain resulting into free radical chain reaction producing peroxyl radicals [41].<br>Another way to generate lipid peroxides is through the attack on polyunsaturated fatty acids (PUFA) or their side chain by the singlet oxygen which is a very reactive form of oxygen [41]. |

## 3. ANTIOXIDANTS

Antioxidants are substances that neutralize free radicals or their actions [42]. The antioxidants acting in the defense systems act at different levels such as preventive, radical scavenging, repair and de novo, and the fourth line of defense, i.e., the adaptation. The first line of defense is the preventive antioxidants, which suppresses the formation of free radicals. The second line of defense is the antioxidants that scavenge the active radicals to suppress chain initiation and/or break the chain propagation reactions. The third line of defense is the repair and de novo antioxidants. The enzymes present in the cytosol and in the mitochondria of mammalian cells recognize, degrade, and remove oxidatively modified proteins and prevent the accumulation of oxidized proteins. There is another important function called adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site [43]. Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic.

### 3.1 Enzymatic antioxidants

Nature has endowed each cell with adequate protective mechanisms against harmful effects of free radicals. Cellular antioxidant enzyme systems serve to protect cells and organisms from the lethal effects of excessive ROS formation. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and glutathione reductase are examples of some antioxidant enzymes.

In eukaryotic cells, O2•- can be metabolized to hydrogen peroxide by two metal containing SOD isoenzymes, tetrameric Mn-SOD present in mitochondria, and dimeric Cu/Zn-SOD present in the cytosol [43, 44]. In the reaction catalyzed by SOD, two molecules of superoxide form hydrogen peroxide and molecular oxygen and are thereby a source of cellular hydrogen peroxide. In mitochondria, superoxide is formed in relatively high concentrations due to the leakage of electrons from the respiratory chain. Expression of Mn-SOD is, in contrast to Cu/Zn-SOD, induced by oxidative stress [44]. Cytosolic Cu/Zn-SOD seems less important than Mn-SOD, and transgenic animals lacking this enzyme are able to adapt so that the phenotype appears normal [45].

Catalases of many organisms are mainly heme-containing enzymes [46]. The predominant subcellular localization in mammalian cells is in peroxisomes, where catalase catalyzes the dismutation of hydrogen peroxide to water and molecular oxygen. Catalase also has functions in detoxifying different substrates, e.g., phenols and alcohols, via coupled reduction of hydrogen peroxide. One antioxidative role of catalase is to lower the risk of hydroxyl radical formation from $H_2O_2$ via the Fenton reaction catalyzed by Cu or Fe ions. Catalase binds NADPH, which protects the enzyme from inactivation and increases its efficiency [47].

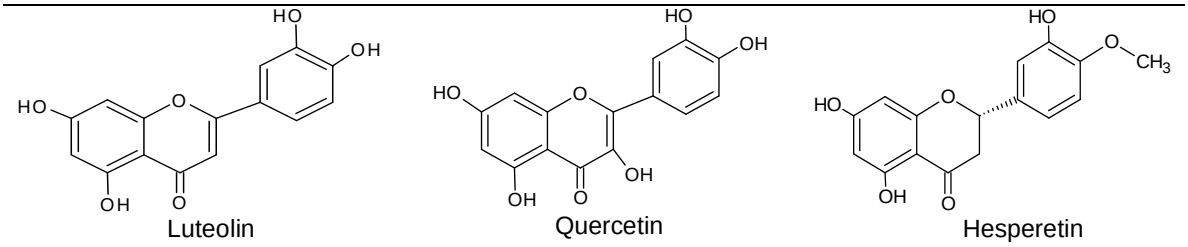
Peroxiredoxins (Prx; thioredoxin peroxidases) are recently discovered enzymes capable of directly reducing peroxides, e.g., hydrogen peroxide and different alkyl hydroperoxides [48]. In mammalian cells, thioredoxin regenerate oxidized Prx formed in the catalytic cycle [49]. In the mitochondria of mammalian cells the mitochondrial thioredoxin system is probably a specific reductant of Prx [50]. Peroxiredoxins have been shown to inhibit apoptosis induced by p53 and by hydrogen peroxide on a level upstream of bcl-2 [51].

There are at least four different Glutathione peroxidases (GPx) in mammals (GPx1–4), all of them

containing selenocysteine [52]. GPx1 and GPx4 both are cytosolic enzymes abundant in most tissues. GPx4 has recently been found to have dual functions in sperm cells by being enzymatically active in spermatids but insoluble and working as a structural protein in mature spermatozoa [53]. GPx2 (gastrointestinal GPx) and GPx3 (plasma GPx) are mainly expressed in the gastrointestinal tract and kidney, respectively [54]. All glutathione peroxidases may catalyze the reduction of $H_2O_2$ using glutathione as substrate. They can also reduce other peroxides (e.g., lipid peroxides in cell membranes) to alcohols. Some data has indicated that GPx should be of high antioxidant importance under physiological conditions while others place the enzymes as important only at events of oxidative stress [55]. The function of GPx isoenzymes in antioxidant defense is still unclear, but the kinetic properties and widespread distribution still imply that they constitute major contributors to the total protection against oxidative damage.

### 3.2 Non enzymatic antioxidants

The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and polyphenols which are obtained from natural plant sources [56]. Some non enzymatic antioxidants are shown in Figure 2. Exposure to DNA by irradiation or hydroxyl radical may leads to the formation of 8-hydroxydeoxyguanosine. On this basis Fischer-Nielsen et al. (1992) [57] found that vitamin C at physiological concentration exhibits a protective effect against free radical-induced oxidative damage. Vitamin E and tocotrienols (such as those from palm oil) are efficient lipid soluble antioxidants that function as a chain breaker during lipid peroxidation in cell membranes and various lipid particles including LDL [58, 59]. Animal studies have shown the antioxidant effect of dietary phytochemicals. Among them, phenolic compounds, such as flavonoids exhibit potent antioxidant activities. For example tea polyphenols have capability to enhance red blood cell resistance to oxidative stress; scavenge superoxide and hydroxyl radicals; and inhibition of oxidative modification of low density lipoprotein. Dietary supplementation of polyphenols is also reported to decrease serum concentrations of total cholesterol and malondialdehyde [21]. β-Carotene and other carotenoids (α-carotene, γ-carotene, and β-cryptoxanthin) are potent antioxidants of plant origin. They react with a peroxyl radical to form a resonance-stabilized carbon-centered radical within its conjugated alkyl structure, thereby inhibiting the chain propagation effect of ROS. Lycopene, lutein, canthaxanthin, and zeaxanthin also have their antioxidant actions similar to those of β –carotene [60]. A wide range of antioxidants from both natural and synthetic origin have been proposed for use in the treatment of various human diseases [61]. Some synthetic antioxidant compounds commonly used in processed foods have been shown to produce toxic effects like liver damage and mutagenesis [5, 62]. Hence, nowadays search for natural compounds antioxidant source is gaining much importance.



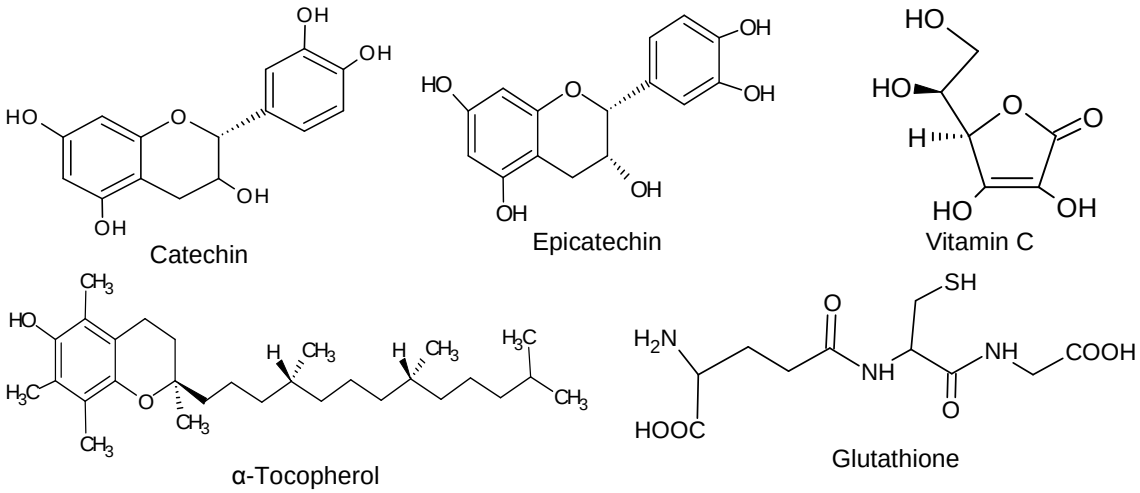



**Fig.2.** Non enzymatic antioxidants

Antioxidant-based drugs/formulations for prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease (AD), Parkinson's disease, cancer, etc. appeared over the past three decades. There are a number of epidemiological studies that have shown inverse correlation between the levels of established antioxidants/phytonutrients present in tissue/blood samples and occurrence of cardiovascular disease, cancer or mortality due to these diseases.

## 4. FREE RADICALS AND HUMAN DISEASES

Free radicals have different types of reaction mechanisms. They can react with surrounding molecules by (a) electron donation, reducing radicals, and electron acceptance, oxidizing radicals, (b) hydrogen abstraction, (c) addition reactions, (d) self-annihilation reactions, and (e) by disproportionation [63]. These reactions lead to the production of ROS, RNS and other radicals which have been linked to many severe diseases like cancer, cardiovascular diseases including atherosclerosis and stroke, neurological disorders, renal disorders, liver disorders, hypertension, rheumatoid arthritis, adult respiratory distress syndrome, auto-immune deficiency diseases, inflammation, degenerative disorders associated with aging, diabetes mellitus, diabetic complications, cataracts, obesity, autism, alzheimer's, parkinson's and huntington's diseases, vasculitis, glomerulonephritis, lupus erythematous, gastric ulcers, hemochromatosis and preeclampsia, among others [64, 65]. Effects of free radicals on disease occurrence are shown below (Fig. 3).

### 4.1 Cancer

DNA is a major target of free radical damage. The types of damages induced include strand breaks (single or double strand breaks), various forms of base damage yielding products such as 8-hydroxyguanosine, thymine glycol or abasic sites, damage to deoxyribose sugar as well as DNA protein cross links. These damages can result in mutations that are heritable change in the DNA that can yield cancer in somatic cells or foetal malformations in the germ cells.

**Table 2.** Radical overload diseases leading to high cancer risk

| Disease | Cancer |
|---|---|

| | |
|---|---|
| Crohn's disease<br>Ulcerative colitis | Colon [67, 68] |
| Barrett's oesophagus | Oesophageal [69] |
| Pancreatitis | Pancreatic [70] |
| Prostatitis | Prostate [71] |
| Human papilloma virus infection | Cervix [72] |
| Viral hepatitis B and C<br>Haemochromatosis | Liver [73, 74] |

The involvement of free radicals with tumor suppressor genes and proto-oncogenes suggest their role in the development of different human cancers [66]. Cancer develops through an accumulation of genetic changes. Initiating agents can be tobacco smoking and chewing, UV rays of sunlight, radiation, viruses, chemical pollutants, etc. Promoting agents include hormones (androgens for prostate cancer, estrogens for breast cancer and ovarian cancer). Inflammation induces iNOS (inducible nitric oxide synthase) as well as COX and LOX. These can initiate carcinogenesis. Table 2 summarizes examples of radical over load diseases. These develop from condition of chronic inflammation and can have an etiology that is primarily inherited or acquired through viral, bacterial and parasitic infection, or acquired through chemical induction. Cancer proneness is frequently a pathological consequence of extensive and sustained free radical stress related damage in these diseases.

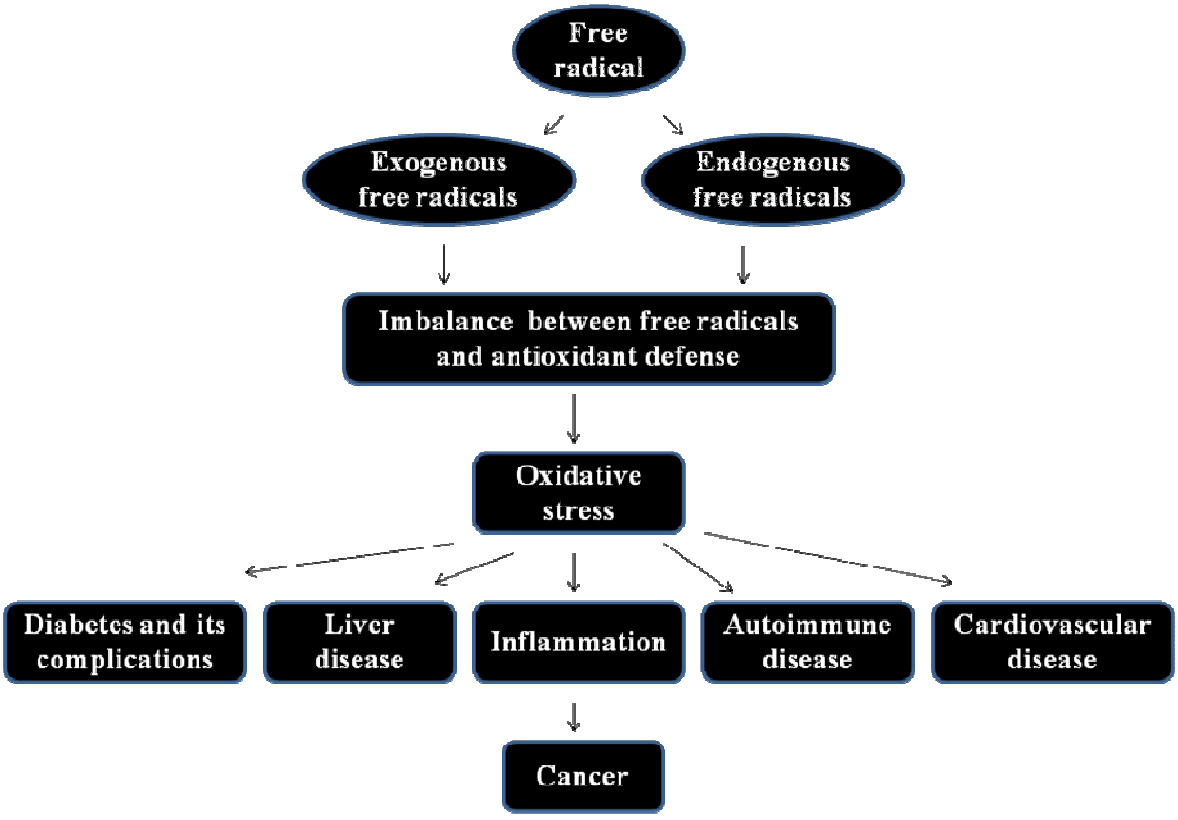


**Fig. 3.** Consequences of free radical load

Experimental as well as epidemiological data indicate that a variety of nutritional factors can act as antioxidants and inhibit the process of cancer development and reduce cancer risk. Some of these include vitamins A, C, E, beta-catotene, and micronutrients [75]. Chemopreventive phytochemicals can block initiation or reverse the promotion stage of multistep carcinogenesis. They can also halt or retard the progression of precancerous cells into the malignant ones. Many molecular alterations associated with carcinogenesis occur in cell-signalling pathways that regulate cell proliferation and differentiation. One of the central components of the intracellular signaling network that maintains homeostasis is the family of mitogen activated protein kinases (MAPKs), they are prime targets of diverse classes of chemopreventive phytochemicals [76]. A number of plants (Table 3) have been found to inhibit cancer progression.

**Table 3:** Phytoconstituents and anti cancer activity

| Plant | Family | Compound | Mode of action |
|---|---|---|---|
| *Catharanthus roseus* | Apocynaceae | Vindesine and Vinorelbine | mitotic block [77] |
| *Catharanthus roseus* | Apocynaceae | Vinflunine | mitotic block [78] |
| *Podophyllum peltatum* | Berberidaceae | Etoposide | mitotic block [79] |
| *Camptotheca acuminate* | Nyssaceae | Topotecan | DNA topoisomerase I inhibition [80] |
| *Berberis amarensis* | Berberidaceae | Berbamine | Caspase-3- dependent apoptosis [81] |
| *Hvdrastis canadensis* | Ranunculaceae | Berberine | Inhibit bcr/abl gene fusion [82] |
| *Tabebuia avellanedae* | Bignoniaceae | Betalapachone | Inhibition of topoisomerase I and II [83] |
| *Betula alba* | Betulaceae | Betulinic acid | Triggers mitochondrial pathway of apoptosis [84] |
| *Colchicum autumnale* | Colchicaceae | Colchicine | Anti-mitotic [85] |
| *Curcuma longa* | Zingiberaceae | Curcumin | Exact mechanism of action is still unknown [86] |
| *Wikstroemia indica* | Thymelaeaceae | Daphnoretin | suppression of protein and DNA synthesis [87] |
| *Psoralea corylifolia* | Fabaceae | Psoralidin | enhanced TRAIL-induced (Tumor necrosis factor-related apoptosis-inducing ligand) apoptosis [88] |
| *Vicia faba* | Fabaceae | Diadzein and Genistein | Inhibits 3A 4- mediated metabolism and oxidative metabolism [89] |
| *Ochrosia borbonica* | Apocynaceae | Ellipticine | DNA intercalation and inhibition of topoisomerase II [90] |

| | | | |
|---|---|---|---|
| *Amoora rohituka* | Meliaceae | Flavopiridol | Inhibits cell cycle progression at G1 or G2 phase [91] |
| *Cephalotaxus harrintonia* | Cephalotaxaceae | Harringtonine | Inhibition of protein synthesis and chain elongation during translation [92] |
| *Ipomoeca batatas* | Convolvulaceae | 4-Ipomeanol | cytochrome P-450 mediated conversion into DNA-binding metabolites [93] |
| *Iridaceaelatea pallasii* | Iridaceae | Irisquinone | Acts as a chemosensitizer[94] |
| *Erythroxylum pervillei* | Erythroxylaceae | Pervilleines | Inhibitors of Pglycoprotein [95] |
| *Salvia prionitis* | Lamiaceae | Salvicine | Inhibition of topoisomerase II [96] |
| *Aglaia foveolata* | Meliaceae | Silvestrol | apoptosome/ mitochondrial pathway is involved in triggering extrinsic pathway of programmed cell death of tumor cells [97] |

## 4.2 Cardiovascular disease

Several established risk factors for cardiovascular disease have been linked to excessive generation of ROS. For instance, in animal models of hiperlipidemia, hypertension, and diabetes, the elevated levels of vascular superoxide anion production have been found [98, 99]. The studies strongly suggest that increased oxidative stress is involved in the pathophysiology of cardiovascular disease. Several mechanisms have been proposed to explain how excessive production of ROS leads to vascular pathology. First, ROS are able to promote the oxidation of low-density lipoprotein (LDL) [100]. Uptake of oxidatively modified lipoproteins by macrophages transforms these cells into foam cells, which are a key component of atherosclerotic plaques [101]. Second, superoxide anion rapidly inactivates endothelium derived nitric oxide (NO), a molecule with intrinsic antiatherogenic properties, leading to endothelial dysfunction, which is a hallmark of early atherosclerosis [102]. Moreover, the reaction between superoxide anion and NO generates peroxynitrite (ONOO-), which has been found to be cytotoxic to endothelial and vascular smooth muscle cells through a broad range of biological actions, such as lipid oxidation and mitochondrial DNA damage. Third, ROS have been shown to be involved in increased expression of certain vascular pro-inflammatory genes that are pertinent to atherogenesis, such as monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) [103, 104].

Phytochemicals prevent endothelial dysfunction and reduce blood pressure, oxidative stress, and end organ damage in hypertensive animals. Moreover, some clinical studies have shown that phytochemicals can improve endothelial function in patients with hypertension and ischemic heart disease [105]. The effects of individual plant products on the relaxation of isolated arteries from rats have been investigated in many studies. Tetracyclic triterpene saponins, the ginsenosides are often attributed to the effects of *Panax ginseng* (Araliaceae) on the cardiovascular system. Studies show that phytosterols also have

effect on the cardiovascular system by lowering cholesterol levels [106].

### 4.3 Diabetes

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated [107]. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [108]. People suffering from diabetes are not able to produce or properly use insulin in the body and therefore chronic hyperglycemia occurs. Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals [109]. Auto-oxidation of glucose involves spontaneous reduction of molecular oxygen to superoxide and hydroxyl radicals, which are highly reactive and interact with all biomolecules. They also accelerate formation of advanced glycation end products (AGEs). AGEs such as pyrroles and imidazoles tend to accumulate in the tissue. Crosslinking AGE-protein with other macromolecules in tissues results in abnormalities in the cell and tissue function. Due to protein glycation capacity of antioxidant enzymes is also reduced. Free radicals generated also react with nitric oxide in endothelial cells leading to loss of vasodilation activity. Long lived structural proteins, collagen and elastin, undergo continual non-enzymatic crosslinking during ageing and in diabetic individuals [110]. This abnormal protein crosslinking is mediated by AGEs generated by nonenzymatic glycosylation of proteins by glucose.

Up to now, many kinds of antidiabetic medicines have been developed for the patients and most of them are chemical or biochemical agents aiming at controlling or/and lowering blood glucose to a normal level. Despite the impressive advances in health sciences and medical care, there are many patients who are using alternative therapies alone or complementary to the prescribed medication. Traditional plant remedies or herbal formulations exist from ancient times and are still widely used, despite all the controversy concerning their efficacy and safety to treat hypoglycemic and hyperglycemic conditions all over the world. To date, metformin (a biguanide) is the only drug approved for treatment of type II diabetes mellitus [111]. It is a derivative of an active natural product, galegine, isolated from the plant *Galega officinalis* L. [112]. Table 4 summarizes the herbs with active components having anti diabetic property.

**Table 4:** Anti diabetic activity of plant products

| Plant | Family | Active compounds | Mode of action |
|---|---|---|---|
| *Abelmoschus moschatus* | Malvaceae | Myricetin | enhances glucose utilization to lower plasma glucose with deficient insulin levels. [113] |
| *Achyrocline satureioides* | Asteraceae | Dibenzofuran Achyrofuran | lowers blood glucose levels[114] |
| *Psacalium* | Asteraceae | Maturine | lowers blood glucose levels [115] |

| | | | |
|---|---|---|---|
| *decompositum* | | | |
| *Acourtia thurberi* | Asteraceae | benzoquinone perezone | lowers blood glucose levels [116] |
| *Allium sativum* | Liliaceae | Allicin | decreases the concentration of serum lipids, blood glucose and activities of serum enzymes [117] |
| *Allium cepa* | Liliaceae | S-methyl cysteine sulfoxide | stimulation of insulin secretions and partly due to its antioxidant activity [118] |
| *Bauhinia forficata* | Leguminosae | Kaempferitrin | decreases lipid peroxidation in liver cells [119] |
| *Bryonia alba* | Curcubiaceae | Trihydroxy octadecadienoic acid | restores the disordered lipid metabolism [120] |
| *Caesalpinia ferrea* | Leguminosae | Ellagic acid | ALR2 inhibitor [121] |
| *Dioscorea dumetorum* | Dioscoreaceae | Dioscoretine | Lowers glucose level [122] |
| *Eucalyptus macrocarpa* | Myrtaceae | Macrocarpals (A, B, C and D) | inhibitory activity against porcine lenses ALR2 [123] |
| *Ficus bengalensis* | Moraceae | Leucopelargonidin | serum insulin raising [124] |
| *Galega officinalis* | Leguminosae | Guanidine | blood glucose-lowering activity[125] |
| *Gentiana olivieri* | Gentianaceae | Isoorientin | Antihyperlipidemic [126] |
| *Hydnocarpus wightiana* | Arcariaceae | Hydnocarpin | alpha-glucosidase and moderate N-acetyl-beta- D-glucosaminidase inhibitory activities [127] |

### 4.4 Oxidative stress and metabolic changes in the liver

Hepatocyte plays a central role in the metabolism of alcohol or drugs which may enhance the ROS production [128]. Under some consequences a large amount of free fatty acids (FFAs) from the visceral fat tissue, as well as from dietary glucose and fat, flows directly into the liver [129]. Due to these mitochondria, peroxisomes, and endoplasmic reticulum metabolize the excessive amount of fatty acid, resulting in overproduction of ROS and oxidative stress in the hepatocytes. Excessively high levels of iron are stored in the hepatocytes of patients with fatty liver, alcoholic hepatitis, or hepatitis type C. Such over accumulation of iron also causes oxidative stress in the hepatocytes [8]. The reason hepatocytes have the highest antioxidant function as compared with the cells of other organs is probably that oxidative stress is easily induced in the hepatocytes.

Herbal medicines have been used in the treatment of liver diseases for a long time. A number of herbal preparations are available in the market. Some commonly used herbal preparations are *Phyllanthus*,

*Silybum marianum* (milk thistle), glycyrrhizin (licorice root extract), and Liv52 (mixture of herbs). *Phyllanthus* appears to be promising in patients with chronic hepatitis B virus (HBV) infection [130]. Liu *et al.* (2001) [131] published a meta-analysis of the effect on and safety of genus *Phyllanthus* for chronic HBV infection. None of the trials reported mortality or incidence of liver cirrhosis and/or hepatocellular carcinoma. *Phyllanthus* has a positive effect on clearance of HBV markers. There are no major adverse effects. Though the active compound remains to be identified, significant progress has already taken place in standardization of the extract to ensure the bioefficacy of *P. amarus* [132].

*Silybum marianum* is the most well researched plant in the treatment of liver disease. In Roman times, Pliny the El-der (A.D. 77), a noted naturalist, reported that milk thistle was excellent for carrying off bile. Culpeper (1650) described its effectiveness in removing obstruction of the liver and spleen [133]. The active complex in mile thistle is a lipophilic extract from the seeds of the plant and is composed of three isomer flavonolignans-silybin, silydianin, and silychrstine collectively known as silymarin [134]. Silymarin acts as an antioxidant by reducing free radical production and lipid peroxidation, has antifibrotic activity, and may act as a toxin blockade agent by inhibiting binding of toxins to heptocyte cell membrane receptors [135]. In animals, silymarin reduces liver injury caused by acetaminophen, carbon tetrachloride, radiation, iron overload, phenylhydrazine, alcohol, cold ischemia, and *Amanita phalloides* [136].

Glycyrrhizin is an aqueous extract of the licorice root, *Glycyrrhizin glabra*. Its major constituents are glycyrrhetic acid, multiple flavonoids, isoflavonoids, hydroxycoumarins and sterols, including *β*-sitosteroid, which may have glucocorticoid and mineralocorticoid activities [137]. Glycyrrhizin prevents several forms of experimental liver injury in animals [138]. This compound has anti-inflammatory and antioxidant activities.

Liv52 is considered to be an Ayurvedic hepatoprotective medicine that contains the *Capparis spinosa* (Himsara), *Cichorium intybus* (Kasani), Mandur bhasma, *Solanum nigrum* (Kakamachi), *Terminalia arjuna* (Arjuna), *Cassia occidentalis* (Kasamarda), *Achillea millefolium* (Biranjasipha), and *Tamarix gallica* (Jhavaka). Liv52 has been on the market for over 50 years and has been claimed to be useful in the prevention and treatment a variety of conditions such as viral hepatitis, alcoholic liver disease, protein energy malnutrition, loss of appetite, and radiation and chemotherapy induced liver damage [139]. Experimental data suggest that Liv52 inhibits lipid peroxidation, may have a protective effect on alcohol induced fetotoxicity, and inhibits TNF activity. Liv52 has been claimed to be useful as an adjuvant to hepatotoxic drugs [140-142].

### 4.5 Free radical and aging

The aging process has been shown to result in an accelerated functional decline. The exact mechanisms that cause this functional decline are unclear. The free radical theory of aging, however, has gained strong support because it is able to explain some of the processes that occur with aging and the degenerative diseases of aging. This theory proposes that an increase in oxygen radical production with age by mitochondria produce an increase in cellular damage [143-145]. Aerobic organisms are well-protected against oxidative challenges by sophisticated antioxidant defense systems. However, it appears

394 that during the aging process an imbalance between oxidants and antioxidants balance may occur.
395 Oxidative damage of biomolecules increases with age and is postulated to be a major causal factor of
396 cellular biochemical senescence [146-148]. Resveratrol, a phytoalexin, is synthesized in the leaf
397 epidermis and the skin (pericarp) of grape berries and has potential antioxidant and anti-aging property
398 [149]. Some plants and their parts having anti aging activity are given in Table 5.
399
400 **Table 5:** Some of the plants and their part used for anti aging activity

| Part used | Plant | Family |
|---|---|---|
| Leaves | *Adansonia digitata* | Bombacaceae [150] |
| | *Alstonia boonei* | Apocynaceae [151] |
| | *Bambusa vulgaris* | Poaceae [152] |
| | *Elaeis guineensis* | Palmae [153] |
| | *Ficus capensis* | Moraceae [154] |
| | *Harungana madagascariensis* | Harungaceae [80] |
| | *Spondias mombin* | Anacardiaceae [155] |
| | *Tectona grandis* | Verbanaceae [156] |
| | *Zea mays* | Poaceae [157] |
| Seed | *Aframomum melegueta* | Zingiberaceae [158] |
| | *Garcinia kola* | Gutiferae [159] |
| Whole plant | *Baphia nitida* | Papilinionaceae [160] |
| | *Lophira alata* | Ochnaceae [161] |
| Root | *Montandra guineensis* | Apocynaceae [162] |
| | *Cocos nucifera* | Palmae [163] |
| Stem bark | *Cordia millenii* | Boraginaceae [164] |
| | *Khaya ivorensis* | Meliaceae [165] |
| Fruits | *Milicia excels* | Moraceae [166] |

401
402 The main function of mitochondria is energy production. During oxidative phosphorylation, however,
403 highly reactive oxygen radicals are generated. One major site of oxidant production occurs in the
404 mitochondrial electron transport chain in which $O_2$ is reduced to $H_2O$. Several studies have investigated
405 age associated increase in the generation of oxidants by mitochondria [167, 168]. Experiments using

intact muscle mitochondria from house flies have shown that the rate of $H_2O_2$ generation progressively increases 2-fold as the house fly ages [169]. The enhanced generation of oxidants by older mitochondria may itself be caused by oxidative damage to mitochondrial membranes and proteins [170]. Miquel and his colleagues have widely promulgated the mitochondrial mutation theory of aging [170]. In this theory, senescence is linked to mutations of mitochondrial DNA (mtDNA) in differentiated cells. Mitochondrial DNA lacks excision and recombination repair mechanisms, it has been postulated that these mutations would lead to problems in replication, leading to a decline in physiological performance and the pathogenesis of many age-related diseases [169, 170]. In addition, mtDNA is not protected by histones or DNA-binding proteins and, therefore, is directly exposed to a high steady state level of reactive oxygen and nitrogen species. Thus, oxidative modification and mutation of mtDNA may occur with great ease. During the aging process, protein oxidation is increased in a wide variety of human and animal tissues. The exact pathways for oxidative cellular damage are poorly understood because the reactive metabolites are very short-lived and difficult to detect directly in vivo. The quantification of oxidative damage to proteins has been studied almost exclusively by assessing the total carbonyl content [171]. The oxidants responsible for carbonyl formation within the proteins in vivo are believed to be radicals, such as, hydroxyl radicals. Indeed, hydroxyl radicals can be generated by metal-catalyzed oxidation systems, and different metal catalyzed oxidation systems convert several amino acid residues to carbonyl derivatives [169-171].

## 5. CONCLUSION AND FUTURE PROSPECTS

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA. Phytochemicals including polyphenols, flavonoids and others have potential to provide defense against oxidative damage. Newer approaches are further required for identification and characterization the specific phytoconstituents from diverse flora for providing protection against oxidative stress.

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