Free Radicals: Health Implications and their Mitigation by Herbals

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Authors' contributions

This work was carried out in collaboration between both authors. Author SK managed the literature searches and prepared the first draft of the manuscript. Author AKP thoroughly reviewed the scientific contents and made desired corrections in the final draft of the manuscript. Both the authors read and approved the final manuscript.

ABSTRACT

Free radicals pose 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

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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.

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 (Fig. 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^-) radicals. The nitrogen derived free radicals are nitric oxide (NO^-) 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 [6-8]. 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 plant 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].

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 O^2 and $O^{2\bullet-}$ [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 to 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].

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, $O_2^{•-}$ 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 Fig. 2. Exposure to DNA by irradiation or hydroxyl radical may leads to the formation of 8-hydroxydeoxyguanosine. On this basis Fischer-Nielsen et al. [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.

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.
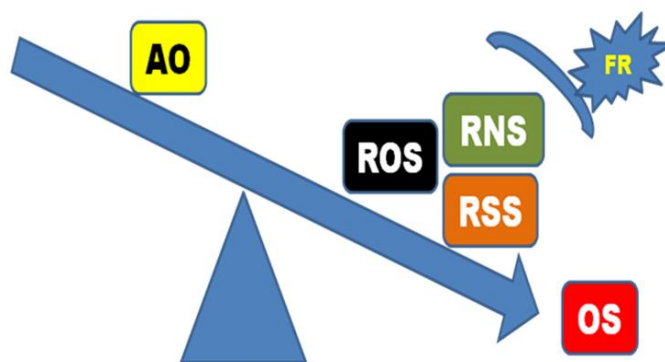

Fig. 1. Effect of imbalance between antioxidants and free radicals
(Abbreviations: AO-antioxidant, ROS-reactive oxygen species, RNS-reactive nitrogen species, RSS-reactive sulphur species, FR-free radicals, OS-oxidative stress)

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

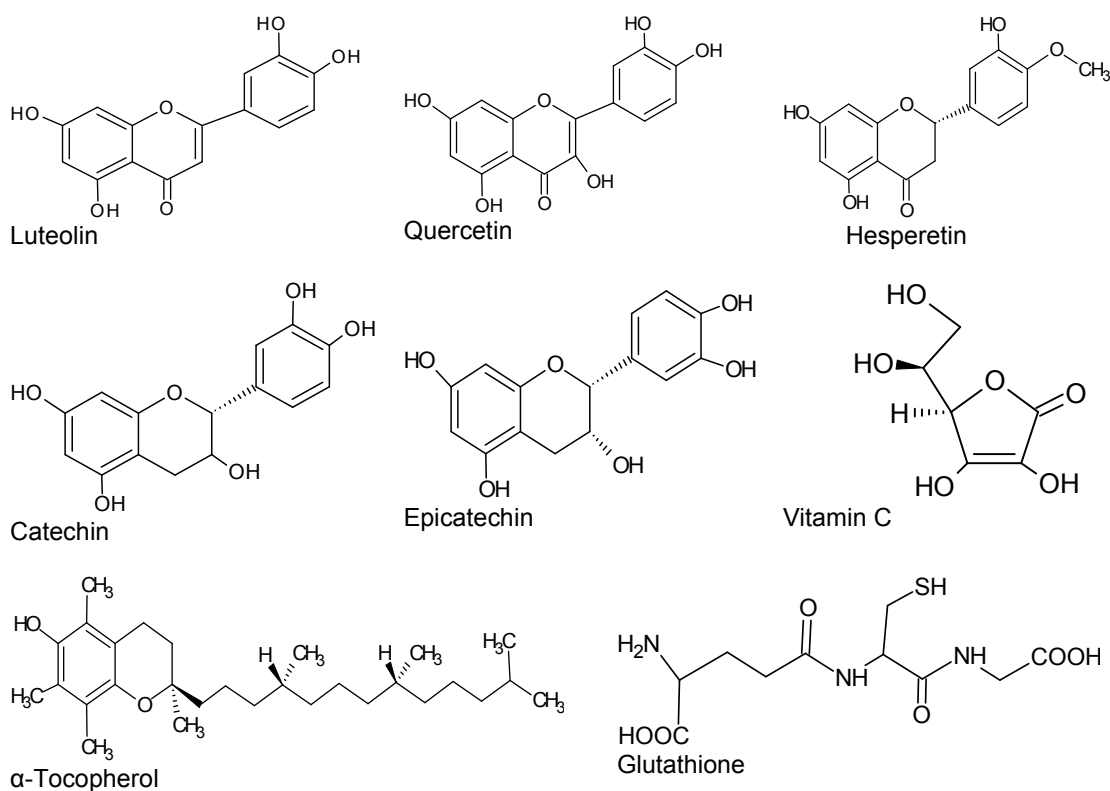| Targets of free radicals	Mode of damage
Proteins	Oxidative modification of a specific amino acid. Free radical-mediated peptide cleavage. Formation of protein cross -linkage due to reaction with lipid peroxidation products [9].
DNA and RNA	Production of base-free sites. Deletions, modification of bases. Frame shifts. Strand breaks. DNA–protein crosslink and chromosomal arrangements. Oxidation of DNA by hydroxyl radicals [39,40].
Sugars	Formation of oxygen free radicals during early glycation could contribute to glycoxidative damage [40]. 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]. 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].

Luteolin

Quercetin

Hesperetin

Catechin

Epicatechin

Vitamin C

α-Tocopherol

Glutathione

Fig. 2. Non enzymatic antioxidants

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.

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.

Table 2. Radical overload diseases leading to high cancer risk

Disease	Cancer
Crohn's disease 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 Haemochromatosis	Liver [73,74]

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.

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].
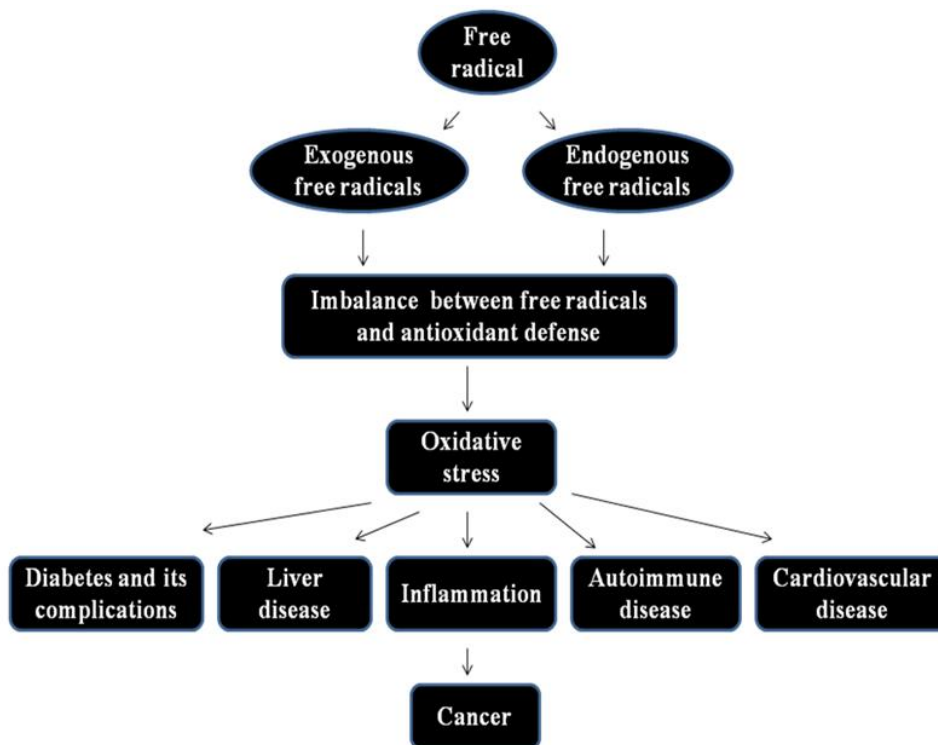

Fig. 3. Consequences of free radical load

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]

4.3 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.

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. [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 [133] described its effectiveness in removing obstruction of the liver and spleen. 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].

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 decompositum	Asteraceae	Maturine	lowers blood glucose levels [115]
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]

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

The main function of mitochondria is energy production. During oxidative phosphorylation, however, highly reactive oxygen radicals are generated. One major site of oxidant production occurs in the mitochondrial electron transport chain in which O_2 is reduced to H_2O. Several studies have investigated 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 [169-173].

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]

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gutteridgde JMC. Free radicals in disease processes: A complication of cause and consequence. Free Rad Res Commun. 1995;19:141-58.
2. Mishra A, Kumar S, Bhargava A, Sharma B, Pandey AK. Studies on *In vitro* antioxidant and antistaphylococcal activities of some important medicinal plants. Cell Mol Biol. 2011;57:16-25.
3. Mishra A, Sharma AK, Kumar S, Saxena AK, Pandey AK. *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant and anticancer activities. BioMed Res Int. 2013(2013); Article ID 810734.
4. Joyce DA. Oxygen radicals in disease. Aust Adv Drug React Bull. 1987;127:476-79.
5. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Sci World J. 2013;2013. Article ID 162750.
6. Kumar S, Sharma UK, Sharma AK, Pandey AK. Protective efficacy of S*olanum xanthocarpum* root extracts against free radical damage: Phytochemical analysis and antioxidant effect. Cell Mol Biol. 2012;58:171-78.
7. Kumar S, Pandey AK. Phenolic content, reducing power and membrane protective activities of *Solanum xanthocarpum* root extracts. Vegetos. 2013;26:301-07.
8. Kumar S, Pandey AK. Antioxidant, lipo-protective and antibacterial activities of phytoconstituents present in *Solanum xanthocarpum* root. Int Rev Biophys Chem. 2012;3:42-47.
9. Kumar S, Chashoo G, Saxena AK, Pandey AK. *Parthenium hysterophorus*: A probable source of anticancer, antioxidant and anti-HIV agents. BioMed Res Int. 2013;2013. Article ID 810734.
10. Pandey AK, Mishra AK, Mishra A. Antifungal and antioxidative potential of oil and extracts derived from leaves of Indian spice plant *Cinnamomum tamala.* Cell Mol Biol. 2012;58:142-147.
11. Pham-Huy, Pham-Huy H He. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4:89-96.
12. Mishra AK, Mishra A, Kehri HK, Sharma B, Pandey AK. Inhibitory activity of Indian spice plant *Cinnamomum zeylanicum* extracts against *Alternaria solani* and *Curvuluria lunata*, the pathogenic dematiaceous moulds. Ann Clin Microbiol Antimicrob. 2009;8:Article 9.
13. Kuppusamya P, Zweier JL. Characterization of free radical generation by xanthine oxidase. Evidence for hydroxyl radical generation. J Biol Chem. 1989;264:9880-84.
14. Zimmerman BJ, Granger DN. Mechanisms of reperfusion injury. Am J Med Sci. 1994;307:284-92.
15. Kontos HA, Ellis EP, Jenkins EF, Povlishock LW, Rowe JT, Hess ML. Appearance of superoxide anion radical in cerebral extracellular space during increased prostaglandin synthesis in cats. Circ Res. 1985;57:142–51.
16. Mcintyre M, Bohr DF, Dominiczak AF.

Endothelial function in hypertension. Hypertension. 1999;34:539-45.

17. Winterbourn CC, Vissers MC, Kettle AJ. Myeloperoxidase. Curr Opin Hematol. 2000;7: 53-58.

18. Lipinski B. Hydroxyl radical and its scavengers in health and disease. Oxid Med Cell Longev; 2011. Article ID:809696

19. Mishra A, Kumar S, Pandey AK. Scientific validation of the medicinal efficacy of *Tinospora cordifolia.* Sci World J. 2013;2013. Article ID 292934.

20. Wu G, Morris SM. Arginine metabolism: nitric oxide and beyond. Biochem J. 1998;336:1-17.

21. Fang Y Z, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nut 2002; 18:872–79.

22. Rassaf T, Preik M, Kleinbongard P. Evidence for in vivo transport of bioactive nitric oxide in human plasma. J Clin Inv. 2002;109:1241-48.

23. Thomas EL, Lehrer RI, Rest RF. Human neutrophil antimicrobial activity. Clin Infect Diseases. 1998;10:S450–S456.

24. Babior B. NADPH oxidase An update. Blood. 1999;93:1464-76.

25. Nauseef WF. The NADPH dependent oxidase of phagocytes. Proc. Assoc. Am. Physicians. 1999;111:373–382.

26. Rossi F, Bellavite P, Berton G, Grzeskowiak M, Papini E. Mechanism of production of toxic oxygen radicals by granulocytes and macrophages and their function in the inflammatory. Path Res Prac. 1985;180:136–42.

27. Rosen HJ, Orman RM, Rakita BR, Michel D, VanDevanter R. Loss of DNA-membrane interactions and cessation of DNA synthesis in myeloperoxidase-treated *Escherichia coli.* Prod Nat Acad Sci USA. 1990;87:10048–10052.

28. Dalton TP, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. Ann Rev Pharmacol Toxicol. 1999;39:67– 101.

29. Babior BM. Superoxide: a two-edged sword. Braz J Med Biol Res. 1997;30:141–55.

30. Arrigo AP. Gene expression and the thiol redox state. Free Rad Biol Med. 1999; 27:936-44.

31. Timblin CR, Janssen YM, Goldberg JL, Mossman BT. GRP78, HSP72/73, and cJun stress protein levels in lung epithelial cells exposed to asbestos, cadmium, or

H_2O_2. Free Rad Biol Med. 1998;24:632–42.

32. Nose KM, Shibanuma, Kikuchi K, Kageyama H, Sakiyama S, Kuroki T. Transcriptional activation of early response genes by hydrogen peroxide in a mouse osteoblastic cell line. Eur J Biochem. 1991; 201:99–106.

33. Valko M, Izakovic M, Mazur M, Rhodes C J, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004;266:37–56.

34. Kumar S, Gupta A, Pandey AK. C*alotropis procera* root extract has the capability to combat free radical mediated damage. ISRN Pharmacol 2013;2013. Article ID 691372.

35. Kumar S, Mishra A, Pandey AK. Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using *In vitro* models. BMC Comp Alt Med. 2013;2013:Article 120.

36. Lu J, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. J Cell Mol Med. 2010;14:840–60.

37. Craft BD, Kerrihard AL, Amarowicz R, Pegg RB. Phenol-based antioxidants and the *In vitro* methods used for their assessment. Compreh Rev Food Sci Food Safety. 2012;11:148–73.

38. Devasagayam TPA, Boloor KK, Ramsarma T. Methods for estimating lipid peroxidation: Analysis of merits and demerits (mini review). Ind J Biochem Biophys. 2003;4:0300-08.

39. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. Free Rad Biol Med. 2002;32:1102-15.

40. Benov L, Beema AF. Superoxide - dependence of the short chain sugars induced mutagenesis. Free Rad Biol Med. 2003;34:429-33 .

41. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nut. 1993;57:715-24 .

42. Pandey AK, Mishra AK, Mishra A, Kumar S, Chandra A. Therapeutic potential o f *C. zeylanicum* extracts: An antifungal and antioxidant perspective. Int J Biol Med Res. 2010;1:228-233.

43. Niki E. Antioxidant defenses in eukaryotic cells, In Free radicals: From basic science

to medicine (Poli G, Albano E, Dianzan M U, eds), Basel, Switzerland: Birkhauser Verlag. 1993;365–373,

44. Yost FJJ, Fridovich I. An iron-containing superoxide dismutase from *Escherichia coli*. J Biol Chem. 1993;248:4905–08.

45. Fridovich I. J Biol Chem. Superoxide anion radical (O_2^{-} '), superoxide dismutases, and related matters. 1997;272:18515-17.

46. Das KC, Lewis-Molock Y, White CW. Am J Res Cell Mol Biol. Elevation of manganese superoxide dismutase gene expression by thioredoxin. 1997;17:713 –26 .

47. Ohlemiller KK, McFadden SL, Ding DL, Flood DG, Reaume AJ, Hffman EK, Scott RW, Wright JS, Putcha GV, Salvi RJ. Targeted deletion of the cytosolic Cu/Zn - superoxide dismutase gene (Sod1) increases susceptibility to noise-induced hearing loss. Aud Neurotol. 1999;4:237–46.

48. Aebi H. In Methods of Enzymatic Analysis (Bergmayer HU, ed) 2nd edn. Academic Press New York, USA. 1974;673-77.

49. Kirkman HN, Gaetani GF. Catalase: A tetrameric enzyme with four tightly bound molecules of NADPH. Prod Natal Acad Sci, USA. 1984;81:4343–47.

50. Chae HZ, Kim HJ, Kang SW, Rhee SG. Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. Diab Res Clin Prac. 1999;45:101–12.

51. Poole LB, Godzik A, Nayeem A, Schmitt J D. AhpF can be dissected into two functional units: Tandem repeats of two thioredoxin-like folds in the N-terminus mediate electron transfer from the thioredoxin reductase -like C-terminus to ahpC. Biochem. 2000;39:6602-6615.

52. Miranda-Vizute ADA, Spyrou G. The mitochondrial thioredoxin system. Ant Redox Signaling. 2000;4:801–81.

53. Zhou YK, Kok H, Chun AC, Wong CM, Wu HW, Lin MC, Fung PC, Kung H, Jin DY. Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. Biochem Biophys Res Commun. 2000;268:921–27.

54. Ursini F, Maiorino M, Brigelius-Flohe R, Aumann K D, Roveri A, Schomburg D, Flohe F. Diversity of glutathione peroxidases. Methods Enzymol. 1995; 252:38–53.

55. Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, Wissing J, Flohe L. Dual function of the selenoprotein PHGPx during sperm maturation. Science. 1999;285:1393–1396.

56. Dreher I, Schmutzler C, Jakob F, Kohrle J. Expression of selenoproteins in various rat and human tissues and cell lines. J Trace Elem Med Bio. 1997;11:83–91.

57. Jones D P, Eklow L, Thor H, Orrenius S. Metabolism of hydrogen peroxide in isolated hepatocytes: Relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H_2O_2. Arch Biochem Biophys. 1981;210:505–16.

58. Lee J, Koo N. In, Reactive oxygen species, aging and antioxidative nutraceuticals. Comphr Rev Food Sci Food Safety. 2004;3:21-33.

59. Fischer-Nielsen A, Poulsen HE, Loft S. 8-Hydroxydeoxyguanosine in vitro: Effects of glutathione, ascorbate, and 5-aminosalicylic acid. Free Rad Biol Med. 1992;13:121-26.

60. Packer L, Ong ASH. Eds. Mechanisms and Health Effects. AOCS Biological Oxidants and Antioxidants, Molecular Press, Champaign; 1998.

61. Kagan VE, Kisin ER, Kawai K, Serinkan B F, Osipov B N, Sertbinova E A, Wolinsky I, Shvedova AA. Towards mechanism based antioxidant interventions. Ann NY Acad Sci. 2002;959:188-98.

62. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Chem Soc.1998;75:199-212.

63. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. Pharmacol Rev. 2001;53:135-59.

64. Wichi HP. Enhanced tumour development by butylated hydroxyanisole (B HA) from the prospective of effect on fore stomach and oesophageal squamous epithelium. Food Chem Toxicol. 1998;26:717-23.

65. Slater TF. Free-radical mechanis ms in tissue injury. J Biochem. 1984;222:1-15.

66. Rahman K. Studies on free radicals, antioxidants and cofactors. Clin Interven Aging. 2007;2:219-36.

67. Singh AK, Gupta A, Mishra AK, Gupta V, Bansal P, Kumar S. Medicinal plant for curing Alzheimer's disease. Int J Pharmaceut Biol Arch. 2010;1:108-14.

68. Halliwell B, Aruoma OI. Eds. DNA and Free Radicals. Boca Raton Press; 1993.

69. Gillen CD, Prior P, Andrews HA, Allan RN. Ulcerative colitis and Crohn's disease: A

comparison of the colorectal cancer risk in extensive colitis. J Gasteroenterol. 1994; 35:1590-92.

70. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer: A population -based study. New Eng J Med. 1990;323:1228-33.

71. Streitz JM. Barrett's esophagus and esophageal cancer. Chest Sur Clin North Am. 1994;4:227-40.

72. Bansal P, Sonnenberg A. Pancreatitis is a risk factor for pancreatic cancer. Gastroenterol. 1995;109:247-51 .

73. Dennis LK, Lynch CF, Torner JC. Epidemiologic association between prostatitis and prostate cancer. Journal of Urology. 2002;60:78– 83.

74. Mitchell H, Drake M, Medley G. Prospective evaluation of risk of cervical cancer after cytological evidence of human papilloma virus infection. Lancet. 1986;15:573-575.

75. Hai-rim S, Chae-un L, Hyung-jong P, Sang-young S, Jungmyeong C, Ha-chin C, Yoonok A, Takao S. Hepatitis B and C Virus, *Clonorchis sinensis* for the risk of liver cancer: A case-control study in Pusan, Korea. Int J Epidemiol. 1996;25:933-40.

76. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HA, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. New Eng J Med. 1985;313:1256-62.

77. Croce CM. How can we prevent cancer? Prod Natl Acad Scie USA. 2001;98:10986-88.

78. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nature Rev Cancer. 2003;3:768-80.

79. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol. 2005;100:72-79 .

80. Okouneva T, Hill BT, Wilson L, Jordan M A. The effects of vinflunine, vinorelbine, and vinblastine on centromere dynamics. Mol Cancer Ther. 2003;2:427-436 .

81. Simoens C, Lardon F, Pauwels B, De Pooter CMJ, Lambrechts HAJ, Pattyn GGO, Breillout F, Vermorken JB. Comparative study of the radiosensitising and cell cycle effects of vinflunine and vinorelbine, *in-vitro.* BMC Cancer. 2008;8:65.

82. Shoeb M, MacManus SM, Jaspars M, Trevidadu J, Nahar L, Thoo-Lin PK, Sarker SD. Montamine, a unique dimeric indole alkaloid, from the seeds of *Centaurea montana* (*Asteraceae*), and its in-vitro cytotoxic activity against the CaCo2 colon cancer cells. Tetrahedron. 2006;62:11172-11177.

83. Creemers GJ, Bolis G, Gore M, Scarfone G, Lacave AJ, Guastalla JP, Despax R, Favalli G, Kreinberg G, Van Belle S. Topotecan, an active drug in the second - line treatment of epithelial ovarian cancer: Results of a large European phase II study. J Clin Oncol. 1996;14:3056-61.

84. Xu R, Dong Q, Yu Y, Zhao X, Gan X, Wu D, Lu D, Xu X, Yu X F. Berbamine: a novel inhibitor of bcr/abl fusion gene with potent anti-leukemia activity. Leukemia Res. 2006;30:17-23.

85. Lei H, Wang B, Li WP, Yang Y, Zhou AW, Chen MZ. Aging effect of astragalosides and its mechanism of action. Acta Pharmacol Sinica. 2003;24:230-34.

86. Li Y, Li C J, Yu D, Pardee AB. Potent induction of apoptosis by β-lapachone in human multiple myeloma cell lines and patient cells. Mol Med. 2000;6:1008-15.

87. Fulda S. Betulinic acid for cancer treat ment and prevention. Int J Mol Sci. 2000; 9:1096-107 .

88. Dubey KK, Ray AR, Behera BK. Production of demethylated colchicines through microbial transformation and scale-up process development. Process Biochem. 2008;43:251-57.

89. Sa G, Das T, Banerjee S, Chakraborty J. Curcumin: From exotic spice to modern anticancer drug. Al Ameen J Med Sci. 2010;3:21-37.

90. Bronikowska J, Szliszka E, Jaworska D, Czuba Z P, Krol W. The Coumarin Psoralidin Enhances Anticancer Effect of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL). Molecules. 2012; 17:6449-6464.

91. Kaufman PB, Duke JA, Brielmann H, Boik J, Hoyt JE. A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: implications for human nutrition and health. J Alt Comp Med. 1997;3:7-12.

92. Moon YJ, Wang X, Morris ME. Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. Toxicol *in vitro*. 2006;20:187-210.

93. Kuo YC, Kuo PL, Hsu YL, Cho CY, Lin CC. Ellipticine induces apoptosis through p53-dependent pathway in human

hepatocellular carcinoma Hep G2 cells. Life Sci. 2006;78:2550-57.

94. Mans DRA, Da Rocha AB, Schwartsmann G. Anti-cancer drug discovery and development in Brazil: Targeted plant collection as a rational strategy to acquire candidate anti-cancer compounds. Oncol. 2000;5:185-98.

95. Ancuceanu RV, Istudor V. Pharmacologically active natural compounds for lung cancer. Alt Med Rev. 2004;9:402-19.

96. Hazra B, Sarma MD, Sanyal U. Separation methods of quinonoid constituents of plants used in oriental traditional medicines. J Chromatography B. 20042; 812:259-75 .

97. Mi Q, Cui B, Silva GL, Lantvit D, Lim E, Chai H, You M, Hollingshead M J, Mayo J G, Kinghorn AD. Pervilleine A, a novel tropane alkaloid that reverses the multidrug resistanc Phenotype. Cancer Res. 2001;61:4030-4037.

98. Deng F, Lu JJ, Liu HY, Lin LP, Ding J, Zhang J. Synthesis and antitumor activity of novel salvicine analogues. Chinese Chem Lett. 2011;22:25-28.

99. Kinghorn D, De Blanco EJC, Chai HB, Orjala J, Farnsworth J R, Soejarto D D, Oberlies NH, Wani NC, Kroll DJ, Pearce CJ. Discovery of anticancer agents of diverse natural Origin. Pure Applied Chem. 2009;81:1051-63.

100. Miller FJ, Gutterman DD, Rios CD, Heistad DD, Davidson BL. Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. Cir Res. 1998;82:1298–1305.

101. Morawietz H, Weber M, Rueckschloss U, Lauer N, Hacker A, Kojda G. Up regulation of vascular NAD(P)H oxidase subunit gp91phox and impairment of the nitric oxide signal transduction pathway in hypertension. Biochem Biophys Res Commun. 2001;85:1130–1135.

102. Hiramatsu H, Rosen H, Heinecke JW, Wolfbauer G, Chait A. Superoxide initiates oxidation of low density lipoprotein by human monocytes. Arteriosclerosis. 1987;7: 55-60.

103. Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. Free Rad Biol Med. 1996;20:707–27.

104. Darley-Usmar V, Wiseman H, Halliwell B. Nitric oxide and oxygen radicals: A question of balance. FEBS Lett. 1995;369:131–35.

105. Chen YN, Chang YJ, Jiang MJ. Monocyte chemotactic protein-1 gene and protein expression in atherogenesis of hypercholesterolemic rabbits. Atherosclerosis. 1999;143:115–23.

106. Boring L, Gosling J, Cleary M, Charo I F. Decreased lesion formation in CCR2 -/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature. 1998;394:894–97.

107. Perez-Vizcaino F, Ibarra M, Cogolludo A L, Duarte J, Zaragoza-Arnaez F, Moreno L, Lo´pez-Lo´pez G, Tamargo J. Endothelium-independent vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries. J Pharmacol Exp The. 2006;302:66–72.

108. Klingberg S, Ellegård G, Johansson I, Hallmans G, Weinehall L, Andersson H, Winkvist A. Inverse relation between dietary intake of naturally occuring plant sterols and serum cholesterol in northern Sweden. Am J Clin Nut. 2008;87:993-1001.

109. Paik SG, Blue ML, Fleischer N, Shin S. Diabetes susceptibility of BA LB/cBOM mice treated with streptozotocin. Inhibition by lethal irradiation and restoration by splenicly mphocytes. Diabetes. 1982; 31:808–15.

110. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old Paradigm. Diabetes. 1999;48:1-9 .

111. Kawamura M, Heinecke JW, Chait A. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide dependent pathway. J Clin Interven. 1984;94:771–78.

112. Vasan S, Foiles P, Founds H. Therapeutic potential of breakers of advanced glycation end products -protein crosslinks. Arch Biochem Biophys. 2009;419:89-96.

113. Huxtable RJ. The harmfull potential of herbal and other plant products. Drug Safety. 1990;5:126-36.

114. Witters L. The blooming of the French lilac. J Clin Interven. 2001;108:1105-07.

115. Liu IM, Liou SS, Lan TW, Hsu FL, Cheng JT. Myricetin as the active principle of Abelmoschus moschatus to lower plasma glucose in STZ-diabetic rats. Planta

Medica. 2005;71:617-21.

116. Caney JR, Krenisky JM, Williamson RT, Luo J. Achyrofuran, a new antihyperglycemicd ibenzofuran from the South A merican medicinal plant *Achyrocline satureioides.* J Nat Prod. 2005;65:203-05.

117. Alarcon-Aguilar FJ, Roman-Ramos R, Jimenez-Estrada M, Reyes-Chilpa R, Gonzalez-Paredes B, Flores-Saenz J L. Effects of three Mexican medicinal plants (*Asteraceae*) on blood glucose levels in healthy mice and rabbits. J Ethnopharmacol. 1997;55:171-77.

118. Alarcon-Aguilar FJ, Hernandez-Galicia E, Campos-Sepulveda AE, Xolalpa-Molina S, Rivas-Vilchis GF, Vazquez-Carrillo LI, Roman-Ramos R. Evaluation of the hypoglycemic effect of *Cucurbita ficifolia* Bouche´ (*Cucurbitaceae*) in different experimental models. J Ethnopharmacol. 2002;82:185-89.

119. Sheela CG, Augusti KT. Antidiabetic effects of S -allylcysteine sulphoxide isolated from garlic *Allium sativum* Linn. Ind J Exp Biol. 1992;30:523-26.

120. Kumari K, Augusti KT. Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (*Allium cepa* Linn) as compared to standard drugs in alloxan diabetic rats. Ind J Exp Biol. 2002;40:1005-09.

121. De Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti M T, Szpoganicz B, Silva FR. Hypoglycemic effect and antioxidant potential of kaempferol-3, 7- O-(alpha)-dirhamnoside from *Bauhinia forficata* leaves. J Nat Prod. 2004;67:829-32.

122. Karageuzyan KG, Vartanyan GS, Agadjanov MI, Panossian AG, Hoult JR. Restoration of the disordered glucose-fatty acid cycle in alloxan-diabetic rats by trihydroxyoctadecadienoic acids from *Bryonia alba*, a native Armenian medicinal plant. Planta Medica. 1998;64:417-22.

123. Ueda H, Kawanishi K, Moriyasu M. Effects of ellagic acid and 2-(2,3,6- t rihydro xy-4-carboxyphenyl) ellagic acid on sorbitol accumulation *In vitro* and *in vivo*. Biol Pharm Bull. 2004;27:1584-87.

124. Iwu MM, Igboko OA, Okunji CO, Tempesta M S. Antidiabetic and ALR2 activities of biflavanones of *Garcinia kola.* J Pharm Pharmacol. 1990;42:290-92.

125. Murata M, Yamakoshi Y, Homma S, Arai K, Nakamura Y. Isolation and characterization of macrocarpals B-G antibacterial compounds from *Eucalyptus macrocarpa.* Bio Biotechnol Biochem. 1992;56:2062-2065.

126. Cherian S, Augusti KT. Antidiabetic effects of a glycoside of leucopelargonidin isolated from *Ficus bengalensis* Linn. Ind J Exp Biol. 1993;31:26-29.

127. Vuksan V, Sievenpiper JL, Koo VY, Francis T, Beljan-Zdravkovic U, Xu Z, Vidgen E. American ginseng (*Panax quinquefolius* L) reduces postprandial glycemia in nondiabetic subjects and subjects with type II diabetes mellitus. Arch Int Med. 2000;160:1009-13 .

128. Ekrem S, Mustafa A, Erdem Y. Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. Life Sciences. 2005;76:1223-38.

129. Reddy SV, Tiwari AK, Kumar US, Rao RJ, Rao JM. Free radical scavenging, enzyme inhibitory constituents from antidiabetic Ayurvedic medicinal plant *Hydnocarpus wightiana* Blume. Phytother Res. 2005; 19:277-81.

130. Morita Y, Ueno T, Sata M. Acta Hepatol Jap. Comparison in liver histology among NASH, ASH and chronic hepatitis type C. 2003;44:A526 .

131. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon, Poynard T. Liver fibrosis in overweight patients. Gastroenterol. 2000;118:1117–1123.

132. Thyagarajan SP, Subramanian S, Thirunaksundari, Venkateswaran PS, Blumberg B S. Effects of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. Lancet. 1998;2:764–66.

133. Liu J, Lin H, McIntosh H. Genus Phyllanthus for chronic hepatitis B virus infection: A systematic review. J Viral Hep. 2001;8:358–66.

134. Thyagrajan SP, Jayaram S, Gopalakrishnan V, Han R, Jayakumar P, Sripathi M S. Herbal medicines for liver diseases in India. J Gastroenterol Hepatol. 2000;17:S370–376.

135. Luper S. A review of plants used in the treatment of liver disease: Part 1. Alt Med Revi. 1998;3:410–21.

136. Feher J, Lang I, Nekam KJ, Gergely P, Muzes G. *In vivo* effect of free radical scavenger hepatoprotective agents on superoxide dismutase (SOD) activity in

patients. Tokai J Exp Clin Med. 1990;15:129–34.

137. Faulstich H, Jahn W, Wieland T. Silybin inhibition of amatoxin uptake in the perfused rat liver. Arzneimittelforschung. 1980;30:452–54 .

138. Jacobs B P, Dennehy C, Ramirej G, Sapp J, Lawrence V A. Milk thistle for the treatment of liver diseases: A systematic review and meta-analysis. Am J Med. 2002;113:506-15.

139. Seef LB, Lindsay KL, Bacon BR, Kresina TF, Hoofnagle JH. Complementary and alternative medicine in chronic liver disease. Hepatol. 2001;34:595-603.

140. Van Rossum TG, Vulto AG, deMan RA, Browner J T, Schalm S W. Review article: Glycyrrhizin as a potential treatment for chronic hepatitis C. Alimentary Pharmacol Therap. 1998;12:199–05.

141. Sandhir R, Gill KD. Hepatoprotective effects of Liv52 on ethanol induced liver damage in rats. Ind J Exp Biol. 1999;37:762–66.

142. Pandey S, Gujrati VR, Shanker K, Singh N, Dhawan KN. Hepatoprotective effect of Liv-52 against CCl4 induced lipid peroxidation in liver of rats. Ind J Exp Bio. 1994;32:674-75.

143. Kumar S, Kumar R, Diwedi A, Pandey AK. *In vitro* antioxidant, antibacterial, cytotoxic activity and *in vivo* efficacy of *Syngonium podophyllum* and *Eichhornia crassipes* leaf extracts on isoniazid induced oxidative stress and hepatic markers. BioMed Res Int. 2014; Article ID 459452:11.

144. Sharma AK, Kumar S, Pandey AK. Cell cycle inhibitory activity of *Piper longum* against A549 cell line and its protective effect against metal-induced toxicity in rats. Ind J Biochem Biophys. 2014;51:358-364.

145. Harman D. Aging: A theory based on free radical and radiation chemistry. J Gerontol. 1956;11:298-300.

146. Harman D. The biologic clock: the mitochondria? J Am Geriatrics Society. 1972;20:145-47.

147. Harman D. Free radicals in aging. Mol Cell Biochem. 1998;84:155-61.

148. Ames BN, Shigenaga MK, Hagen TM. Oxidants, Antioxidants and the degenerative diseases of aging. Prod Natl Acad Sci USA. 1993;79:15-22 .

149. Dean RT, Fu S, Stocker R, Davies MJ. Evidence for roles of radicals in protein oxidation in advanced human atherosclerotic plaque. J Biochem. 1997;324:1-18 .

150. Beckman KB, Ames B. The free radical theory of aging matures. Physiol Rev. 1998;78:547-81 .

151. Orr WC, Sohal RS. Extension of life-span by over expression of superoxide dismutase and catalase in *Drosophila melanogaster.* Science. 1994;263:1128-1130 .

152. Chintawar SD, Somani RS, Kasture VS, Kasture SB. Nootropic activity of *Albizia lebbeck* in mice. J Ethnopharmacol. 2002;81:299-05 .

153. Shiva kumar L, Gouda ST, Venkat RN, Verma R. Evaluation of memory enhancing activity of SR-105 in Experimental animals. Int J Res Ayur Pharm. 2011;2:973-77.

154. Chiba T, Yamaza H, Higami Y, Shimokawa I. Anti-aging effects of caloric restriction: involvement of neuroendocrine adaptation by peripheral signaling. Micro Res Technol. 2002;59:317-24.

155. Bastianetto S, Quirion R. Natural extracts as possible protective agents of brain aging. J Neurol Aging. 2002;23:891-97.

156. Chang IM. Anti-aging and health-promoting constituents derived from traditional oriental herbal remedies: Information retrieval using the TradiMed 2000 DB. Ann N Y Acad Scie. 2000;928:281-6.

157. Yu MS, SaranaKa-Yang L, Sau-Wan L, Chi-Ming C, Sze-Yong Z, Mattson M P. Emerging neuroprotective strategies for Alzheimer's disease: Dietary restriction, telomerase activation, and stem cell therapy. J Exp Gerontol. 2000;35:489-02.

158. Schmid DC, Schürch P, Blum E, Belser F, Zü I. Plant stem cell extract for longevity of skin and hair. Int J App Sci. 2008;134:30-35.

159. Oladele AT, Alade GO, Omobuwajo OR. Medicinal plants conservation and cultivation by traditional medicine practitioners (TMPs) in Aiyedaade Local Government Area of Osun State, Nigeria. Agri Biol J N Am. 2011;2:476-87.

160. Schulz V, Hansel R, Tyler V. Rational phytotherapy: A physician's guide to herbal medicine. 3rd ed, Berlin, Germany: Springer-Verlag. 1998;107-123.

161. Hanumanthachar J, Navneet K, Jyotibala C. Evaluation of nootropic effect of Argyreiaspeciosa in mice. J Health Sci. 2000;53:382-88.

162. Singh HK, Dhawan BN. Neuropsycho

pharmacological effects of the Ayurvedic nootropic *Bacopa monniera* Linn. (Brahmi). Ind J Pharmacol. 1997;29:359-65.

163. Vollala VR, Upadhya SS. Nayak S. Effect of *Bacopa monniera* Linn. (Brahmi) extract on learning and memory in rats: A behavioral study. J Vet Behavior. 2010;5:69-74.

164. Singh PP, Chandra A, Mahdi F, Ray A, Sharma P. Reconvene and reconnect the antioxidant hypothesis in Hu man health and disease. Ind J Clin Biochem. 2010;25:225-43.

165. Sudharani D, Krishna KL, Deval K. Safia A K. Pharmacological profiles of *Bacopa monniera*: A review. Int J Pharmacol. 2011;1:15-23.

166. Deval K, Vaibhav S, Krishna KL. Effect of bacopaon memory deficit produced by chronic admin istration of Topiramatein Rats. Int J Pharmacol. 2011;1:118-24.

167. Hagen TM, Yowe DL, Bartholomew JC, Wehr CM, Do KL, Park JY, Ames BN. Mitochondrial decay in hepatocytes from old rats: Membrane potential declines, heterogeneity and oxidants increase. Prod Natal Acad Sci USA. 1997;94:3064-69.

168. Sastre J, Pallardo FV, Pla R, Pellin A, Juan G, O'Connor JE, Estrela JM, Miquel J, Vina Aging of the liver: Age-associated mitochondrial damage in intact hepatocytes. J. Hepatol. 1996;24:1199-05.

169. Sohal RS. Sohal BH. Hydrogen peroxide release by mitochondria increases during aging. Mec Aging Dev. 1991;57:187-202.

170. Miquel J. Fleming JE. A two-step hypothesis on the mechanisms of *In vitro* cell aging: Cell differentiation followed by intrinsic mitochondrial mutagenesis. Exp Gerontol. 1984;19:31-36.

171. Iquel JM. An update on the oxygen stress - mitochondrial mutation theory of aging: genetic and evolutionary implications. J Exp Gerontol. 1988;33:113-26.

172. Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 1991;233:346-56.

173. Lett BS, Stadtman E. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem. 1997;272:2031-36.

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