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Review Article

IMPORTANCE OF FREE RADICAL AND ANTIOXIDANT ON HUMAN HEALTH

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ABSTRACT

Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful tothebody.Theyareproducedeitherfromnormalcellmetabolismsinsituor from external sources(pollution, cigarette smoke, radiation, medication).When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative illness such as cancer, auto immune disorders, aging, cataract, rheumatoid arthritis, cardio vascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced insitu, or externally supplied through foods and/or supplements. This mini-review deals with the taxonomy, the mechanisms of formation and catabolism of the free radicals, it examines their beneficial and deleterious effects on cellular activities, it highlights the potential role of the antioxidants in preventing and repairing damages caused by oxidative stress, and it discusses the antioxidant supplementation in health maintenance.

In recent years, much attention has been focused on the use of nature preservatives to enhance the quality, safety and stability of ready-to-eat food products. These products also undergo gradual changes during storage, due to autoxidation, which releases reactive oxygen species including free radicals into the food. The consumption of seaweed as food and nutraceutical has been well know in the days, increasingly seaweeds are being investigated for the biological activity of their extracts which are finding hundreds of applications in pharmaceuticals, biotechnology and food preservatives. The present study demonstrates that H.elongata contains excellent antimicrobial and antioxidant properties which can provide opportunities for the application of seaweed extract as natural food preservative or nutraceutical for possible applications in food and dietary supplemental products for health promotion.[2]

Keywords: Free Radicals, Antioxidants, Preservatives, Reactive oxygen species.

1. INTRODUCTION

Reactive oxygen species (ROS) is a collective term used for a group of oxidants, which are either free radicals or molecular species capable of generating free radicals. Intracellular generation of ROS mainly comprises superoxide (O2•−) radicals and nitric oxide (NO•) radicals. Under normal physiologic conditions, nearly 2% of the oxygen consumed by the body is converted into O2•− through mitochondrial respiration, phagocytosis, etc ROS percentage increases during infections,exercise, exposure to pollutants, UV light, ionizing radiation, etc. NO•, is an endothelial relaxing factor and neurotransmitter, produced through nitric oxide synthatase enzymes. NO• and O2•− radicals, are converted to powerful oxidizing radicals like hydroxyl radical (•OH), alkoxy radicals (RO•), peroxyl radicals (ROO•), singlet oxygen ($^1O2$) by complex transformation reactions. Some of the

radical species are converted to molecular oxidants like hydrogen peroxide (H_2O_2), peroxynitrite (ONOO-), hypochlorous acid (HOCl). Sometimes these molecular species act as source of ROS.

For example, H_2O_2 is converted to •OH radicals by Fenton reaction and HOCl through its reaction with H_2O_2 can be converted to $^1O2$. ONOO- at physiological concentrations of carbon dioxide becomes a source of carbonate radical anion (CO_3•-). The various pathways involved in the generation of ROS are given in fig 1.[3]

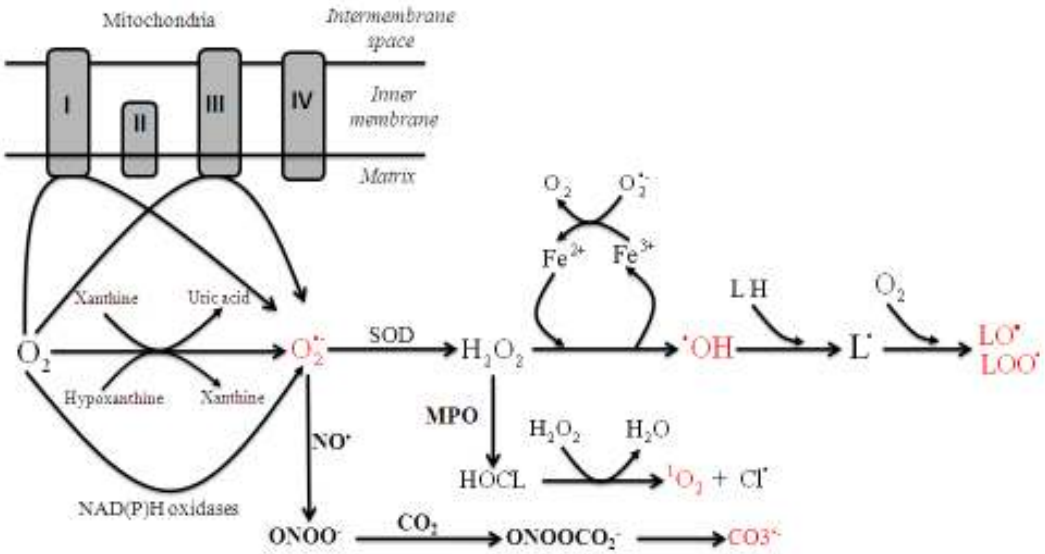

Fig. 1: Production of free radicals via differentroutes[3]

1.1 Reactive oxygen species and free radicals

Reactive oxygen species is a collective term that includes all reactive forms of oxygen, including both radical and nonradicalspeciesthatparticipateintheinitiationa nd/orpropagationofchain reaction. Free radicals represent a class of highly reactive intermediate chemical entities whose reactivity is derived from the presence of unpaired electron in their structure, which are capable of independent existence for very brief interval of time.

Free radicals and other reactive species are derived either from normal essential metabolic processes or from external sources, such as exposure to x-rays, ozone, cigarette smoking, air pollutants, industrial chemicals etc. [4]

1.2 Source of Free Radical

•Internal sources
•External source
•Physiological Factors

1.2.1 Internal sources

These can be enzymatic reactions, which serve as a source of free radicals. These include those reactions involved in respiratory chain,in phagocytosis, in prostaglandin synthesis and in the cytochrome P_{450} system. Some internal sources of generation of free radicals are mitochondria, xanthine oxidase, phagocytes, reactions involving iron and other transition metals, peroxisomes, Arachidonate pathways, exercise, ischaemia/reperfusion, inflammation.

1.2.2 External sources

Theseincludenonenzymaticreactionsoftheoxyge nwithorganiccompounds.Freeradicals also arise in reactions, which are initiated by ionizing radiations. Some external sources of 6 free radicals are cigarette smoke, environmental pollutant, radiations, ultraviolet light, ozone, certain drugs, pesticides, anesthetics and industrial solvents.

1.2.3 PhysiologicalFactors

Mental status like stress, emotion etc. and disease conditions are also responsible for the formation of free radicals.[5]

1.3 Types of Free Radicals.

•Hydroperoxyl radical
•Superoxide radical
•Hydrogen peroxide
•Triplet Oxygen
•Active Oxygen

1.3.1 Hydroperoxyl radical

The hydroperoxyl radical, also known as the perhydroxyl radical, is the protonated form of superoxide with the chemical formula HO_2. Hydroperoxyl is formed through the transfer ofa proton to an oxygen atom. HO_2 canactasanoxididantinanumberof biologically important reactions, such as the abstraction ofhydrogen atomsfromtocopheroland polyunstaturated fattyacids inthe lipidbilayer. Assuch,itmaybeanimportantinitiatoroflipid peroxidation.

1.3.2Superoxide

Superoxide can act either as oxidant or reductant, it can oxidize sulphur, ascorbic acid or NADPH and it can reduce Cytochrome C and metal ions. A dismutation reaction leading to the formation of hydrogen peroxide and oxygen can occur spontaneously or is catalyzed by enzyme superoxide dismutase. In its protonated form (pKa4.8), superoxide forms and perhydroxyl radical, which is powerful oxidant but its biological relevance is probably minor because of its low concentration at physiological pH.

1.3.3 Singlet oxygen

It is not a free radical but it can be formed in some radical reactions and can trigger off others. This arises from hydrogen peroxide molecules. Singlet oxygen on decomposition generates superoxide and hydroxyl radicals.

1.3.4 Triplet oxygen

Triplet oxygen can react with elements and ions to form oxides, but usually not with organic compounds, which are in singlet state. However, it reacts easily with free radical molecules produced by the action of other active radicals, radiations, ultraviolet light, and heat or by complex formation with oxygen and transition metal to produce active peroxide radicals and trigger auto-oxidation of unsaturated fatty acids and others.

1.3.5 Hydrogen peroxide

The univalent reduction of superoxide produces hydrogen peroxide, which is not a free radical because all its electrons are paired. It readily permeates through the membranes and is therefore not compartmentalized in the cell. The main damages caused by this are breaking up of DNA, resulting in single strand breaks and formation of DNA protein crosslink. Numerous enzymes (peroxidases) use hydrogen peroxide as a substrate in oxidation reactions involving the synthesis of complex organic molecule. This is an oxidizing agent but not specially reactive and its main significance lies in it being a source of hydroxyl radical in the presence of reactive transition metal ions.[6]

1.4 Damages caused by free radicals

If free radicals are not in activated, their chemical reactivity and a mageallcellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. Their destructive effect on protein may play a role in the causation of diseases, like cataracts. Free radical damage to DNA is also implicated in the causation of cancer and its effect on LDL cholesterol is very likely responsible for heart disease. Free radicals are also responsible for ageing.

1.4.1 Oxidative damages to proteins

Oxidative attack on proteins results insite-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross linked reaction products, altered electrical charges and increased susceptibility to proteolysis.

1.4.2 Oxidative damage to DNA

Activated oxygen and agents that generate oxygen free radicals, such as ionizing radiations, induce numerous lesions in DNA that causes deletion, mutations and other lethal genetic effects. Characterization of this damage to DNA has indicated that both sugar and base moieties are susceptible to oxidation, causing base degradation, single strand breakage and cross links to proteins.[7]

1.5 Free radical and diseases

Free radicals result in number of human degenerative diseases affecting a wide variety of physiological functions such as atherosclerosis, diabetes, ischemia /reperfusion (l/R) injury, inflammatory diseases (rheumatoid arthritis, inflammatory bowel diseases and pancreatitis), cancer, neurological diseases, hypertension etc. Free radicals are however, not always harmful. They also serve useful purpose in the human body. The oxygen radicals in the living system are probably necessary compounds in the maturation process of cellular structure. White blood cells release free radicals to destroy invading pathogenic microbes as a part of body defense mechanism against diseases. Hence, complete elimination of these radicals would not only be impossible but also harmful.[8]

1.6 Mechanism for the formation of free radicals

Free radicals can be formed by three ways

By homolytic cleavage of covalent bond of normal molecule, with each fragment retaining one of paired electrons.

$$X:Y \rightarrow X^{*}+Y^{*}$$

By the loss of single electron from normal molecule

$$X:Y \rightarrow X^{+}+Y$$

Byaddition of single electron to normal molecule

$$X+e^{-} \rightarrow X^{-}$$

Aradicalmightdonateitsunpairedelectrontoother molecule.Itmighttakeelectron from other moleculeinordertopairoritmightsimplyjointothe molecule.Whenradicalgives one electron or takesoneelectronorsimplyaddsontotheaniontob ecomearadical.Thusthe future of the reactionsthatusuallyproceedaschainreactionissu chthatoneradicalbegets another.[9]

1.7 Antioxidants

Antioxidantsareaninhibitoroftheprocessofoxidat ion,evenatrelatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A varietyoffreeradicalscavengingantioxidantsisfou ndindietarysourceslikefruits,vegetables and tea, etc.

According to literature, these are substance that when present in low concentration compared to those of the oxidisable substrates significantly delay or inhibit the oxidation of that substance.

The antioxidant can also be defined as "A compound capable of inhibiting oxygen mediated oxidation of diverse substances from simple molecule to polymer and complex bio-systems. According to US Food and Drug Administration (FDA), antioxidants are defined as substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation. While antioxidants are of interest to the food industry because they prevent rancidity in food, antioxidants are also of interest to biologist and clinicians because they may help to protect human body against damage by reactive oxygen species (ROS). Antioxidants defense both enzymatic c and non enzymatic reactions protect the body against oxidative damage. Non enzymatic antioxidants are frequently added to the food to prevent lipid oxidation. Several lipid antioxidants can exert pro-oxidant effect towards other molecule under certain circumstances thus the antioxidants for food and therapeutic use must be characterized .[10]

Table 1: Antioxidant components in food[11]

Components	Compounds	Food sources
Vitamins	Vitamin C (ascorbic acid) Vitamin E (tocopherols and tocotrienols, Beta carotene and other carotenoids	Citrus fruit, berries, papaya Seed-like cereal grains, nuts and oils derived from plants, Orange pigmented, and green leafy vegetables.
Elements	Copper (as part of superoxide dismutases), Selenium (as part of glutathione peroxidase)	Cocoa, wheat bran, yeast,Grains, meats
Macronutrient-derived	Peptides e.g. glutathione	Whey protein
Phytochemicals (food components of plant origin)	Isoflavones e.g. genistein and daidzeinFlavonols e.g. quercetin and kaempferol,Polyphenols e.g. rosmarinic acid,Catechins e.g. epigallocatechingallate (EGCG)	Soy Tea, red wine, onions, apples Herbs - oregano, thyme,Green tea
Zoochemicals (food components of animal origin)	Glutathione Ubiquinone (coenzyme Q10)	Meats Meats, especially meat organs, fish

Table 2: Classification of antioxidants[12]

A. CLASSIFICATION BASED UPON THEIR NATURE

1. **Enzymatic antioxidant**

   Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and Glutathione reductase (GR).

2. **Non-enzymatic antioxidant**

   a) ***Metabolic antioxidants***

   Reduced glutathione (GSH), lipoid acid, L-arginine, coenzyme Q_{10}, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc

   b) ***Nutrient antioxidants***

   Vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc

B. CLASSIFICATION BASED UPON SOURCE

1. **Endogenous Antioxidants**

   Bilirubin, glutathione, lipoic acid, *N*-acetyl cysteine, NADPH and NADH, ubiquinone (coenzyme Q_{10}), uric acid, enzymes (SOD, CAT, GPx, GR).

2. **Dietary Antioxidants**

   Vitamin C, Vitamin E, Beta carotene and other carotenoids and oxycarotenoids (lycopene and lutein), polyphenols (flavonoids, flavones, flavonols, and proanthocyanidins

3. **Metal Binding Proteins**

   Albumin (copper), ceruloplasmin (copper), metallothionein (copper), ferritin (iron), myoglobin (iron), transferrin (iron).

C. CLASSIFICATION BASED MECHANISM OF ACTION

1. **Catalytic systems to neutralise or divert ROS**

   SOD, CAT, GPx

2. **Binding/inactivation of metal ions prevents production of ROS by Haber–Weiss reaction**

   Ferritin, caeruloplasmin, catechins

3. **Self suicidal and chain breaking antioxidants scavenge, destroy ROS**

   Vitamin C, vitamin E, uric acid, glutathione, flavonoids

4. **Quenching ROS, chemical traps/sinks to 'absorb' energy**

   Carotenoids, anthocyanidins

1.8 Classification of Antioxidant

A.Natural antioxidants
B. Synthetic antioxidants

1.8.1 Natural antioxidants

Naturally occurring antioxidants of high or low molecular weight can differ in their Composition. Int heir physical and chemical properties, in their mechanism and in their site of action. They can be divided into following categories

1.8.1.1 Enzymes

Enzyme such as superoxide dismutase (SOD), catalase and glutathione peroxidase attenuate the generation of reactive oxygen species by removing potential oxidants or by transferring ROS/RNS (reactive nitrogen species) into relatively stable compounds. SOD which was discovered in late 60s, catalyses the transformation of the superoxide radical into hydrogen peroxide, which can than be transformed by enzyme catalase into water and molecular oxygen. While superoxide anion in itself is not particularly reactive, it can reduce transition metal ions, such as iron and gets converted to most reactive radicals - the hydroxyl radical. Thus, elimination of superoxide radical can attenuate the formation of hydroxyl radical. Glutathione peroxidase (GPx) reduces lipid peroxides (ROOH), formed by the oxidation of polyunsaturated fatty acids (PUFA), to a stable, non toxic molecule - hydroxyl fatty acid (ROH). Together with phosholipase, GPx can also convert phospholipids hydro peroxide (PL-OOH) into phospholipids hydroxide (PL-OH).

1.8.1.2 Low molecular weight antioxidants

These are subdivided into lipid-soluble antioxidants (tocopherol, carotenoids, quinones, bilirubin and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and polyphenols). These delay or inhibit cellular damage mainly through free radical scavenging property.

1.8.1.2Lipids soluble antioxidants

These antioxidants tend to accumulate in lipid plasma lipoprotein (eg. LDL); upon supplementation. This group of antioxidants are supposed to act as highly efficient scavengers, such as against lipid peroxyl radical, which are formed within the Water soluble antioxidants: These antioxidants cannot enter the lipid moiety of low density lipoprotein (LDL); these will be less efficient as these are principally unable to encounter most of these lyophilic radicals; however, such a compound may act in a synergistic manner with lipophilic antioxidants by regenerating them.protein as a consequence of free radical chain reaction

1.8.2 Synthetic antioxidants

These are most effective antioxidants and are synthetic chemicals, approved by Food and Drug Administration for addition to foods, ego BHA (Butylated hydroxy anisole), BHT (Butylated hydroxy toluene).[13]

1.9 Mode of action of antioxidants

In general, the antioxidants act by the following routes

- Chain breaking reaction eg. tocopherol, which act in lipid phase to trap free radical.
- By reducing concentration of reactive oxygen species eg. Glutathione.
- By scavenging initiating radicals ego superoxide dismutase which act in the lipid phase to trap superoxide free radicals.
- By chelating transition metal catalyst: a group of compound which act by sequestration of transition metals that are well established proxidants. In this way transferring, lactoferrin and ferritin function to keep iron induced oxidant stress in check .[14]

1.10 APPLICATIONS OF ANTIOXIDANTS

1) Lanthanides as anti-cancer agents

Howevermuch attention has focused on designing new coordination compound with improved pharmacological properties and a broader range of antitumor activity. Strategies for developing new anticancer agents include the incorporation of carrier groups that can target tumor cells with high specific activity.

2) Lycopene as a potential anti cancer agent

Epidemiology studies have provided evidence that high consumption of tomatoes effectively lowers the risk of reactive oxygen species (ROS)-mediated diseases such as cardiovascular diseases and cancer In addition to its antioxidants properties, lycopene shows an array of biological effects include cardio protective.

3) Selenium derivatives as cancer preventive agents

Consequently, selenium supplementation has moved from the realm of correcting nutritional deficiencies to one of pharmacological intervention, especially in the domain of cancer chemoprevention and in the control of heart failure.

4) Lipoic acid, the antioxidant's antioxidant

Lipoic acid protects against diseases of aging, this offer powerful antioxidant protection lam against three common afflictions (two of them potentially disastrous) association with the aging stroke, heart attack and cataracts. It does it by suppressing the action of free radicals in the cells of the brain, heart and eyes. Lipoid acid has an unusual relationship with four other

important antioxidants: glutathione, coenzymeQ10, vitamin C and vitamin E.[15]

1.11 Uses of Antioxidant

1. Blood purifier, Antimalarial, Antifungal, Anti infertility agent.
2. Antifertilityagent,Expectorant,Diuretic, Carminative,Anthelmentic Bronchitis, Piles,
3. Aphrodisiac, Jaundice, Leprosy, Tumour. Laxative,Antipyretic,Anthelmenti
4. Antidiabetic,Aphrodisiac,Carminative,in Rheumatism.
5. Leukoderma,Scabies,Oxidative stress, Febrifuge,Anthelmentic,Antimalarial, Laxative.
6. Analgesic, Hepatoprotective, Increases immunity.
7. Disorders of digestive tract and kidneys
8. Diuretic, demulcent, emollient
9. In cough and bleeding from lungs.
10. Aphrodisiac, alzheimer, dementia.
11. Demulcent, mildly astringent and expectorant.
12. Heart disease, cancer.[16]

1.12 Limitations of antioxidant supplementation

The primary concern regarding antioxidant supplementation is their potentially deleterious effects on ROS production (pro-oxidant action) especially, When precise modulation of ROS levels are needed to al-low normal cell function 43. In fact, Some negative effects of antioxidants when used in dietary supplements, Mechanistic investigation has revealed that antioxidants may exhibit pro-oxidant activity depending on the specific set of conditions of particular importance are their dosage redox conditions and also the presence of free transition metals in cellular milieu. For example, ascorbate, a well-known antioxidant in the presence of high concentration of ferric iron is a potent mediator of lipid peroxidation. Recent studies suggest that ascorbate sometimes increases DNA damage in humans. Similarly β-carotene also can behave as a pro-oxidant in the lungs of smokers. Of note, natural antioxidant compounds have relatively poor bioavailability. It is therefore necessary to take into cognizance the bio-availability and differential activities of natural and synthetic antioxidant compounds before considering important factors controlling the antioxidant and pro-oxidant activities of curcuma.[17]

1.13 Do antioxidants delay ageing or prevent age-related diseases?

One of the major theories about biological ageing is that it depends on oxidation processes. For this reason, there is great interest in the antioxidant capacity of the human diet and of nutrient supplements. So far, most evidence suggests that plant-derived food is protective against age-related diseases, like cardiovascular disease and cancer, rather than ageing itself.

Many epidemiological studies have linked diets containing moderate to high proportions of fruit and vegetables to lower mortality and to a reduced risk of developing cardiovascular disease, cancers, cataracts and macular degeneration, cognitive impairment and Alzheimer's disease. Although clear cause and effect relationships are difficult to establish, these protective effects are probably due to combinations of nutrients and also to the non-nutritive substances found in these foods. In cohort studies, a survival advantage can be predicted if the diet contains a variety of food, principally from plant sources.[18]

1.14 Can we get enough antioxidant nutrients from food?

Any factors such as excessive dietary fat intake, smoking or alcohol consumption, leading to an increase in oxidation, could increase the requirement for antioxidant nutrients above that usually obtainable from food.

An advantage in getting antioxidants from food is that there are literally thousands of different antioxidants in the human diet and they are numerous in chemical types.

They may therefore act in integrated systems or cascades in which antioxidants may ferry free radicals within the biological system to safer destinations. For example, ROS or RNS may be dissipated from a lipid soluble environment, without lipid peroxide formation, to a water soluble environment throughthe availability of, in sequence, ubiquinone (coenzyme Q10), vitamin E and vitamin C. Upon oxidation, these micronutrients need to be regenerated in the biological setting, hence the need for further coupling to other reducing systems such as glutathione / glutathione disulfide, dihydrolipoate/ lipoate, or NADPH/NADP+ and NADH/NAD+. No one antioxidant can achieve this outcome alone.

Some actually work better when co-ingested in a group of antioxidants. The mix of antioxidants may also facilitate absorption. An example of this is the enhancement of lycopene absorption after taking a combination of beta carotene and lycopene.

According to the National Health and Medical Research Council, Australians have 'access to a nutritious and varied food supply, containing all the known nutrients in more than adequate amounts. People eating a good diet that included breads and cereals, vegetables and fruit, meat or meat substitutes and dairy products do not require vitamin and mineral supplements. These foods, whether fresh or processed, provide a balanced source of vitamins and minerals'.
however, a question as to whether this statement is valid.
This is not because the Australian diet cannot provide enough antioxidant nutrients, but rather whether or not it provides the range and amounts of these nutrients required for optimal health given the current food choices by some groups. For example, according to the 1995 National Nutrition Survey, young Australians do not eat enough fruit as a source of antioxidants; only 37% of those aged 19-24 years reported eating fruit the day before interview.[191.14]

1.14 Are supplements beneficial and safe?
Many believe that if enough of an essential nutrient is good, then more is better. However, when large amounts of antioxidant nutrients are taken, they can also act as pro-oxidants by inducing oxidative stress. Furthermore, pro-oxidant activity can induce either beneficial or harmful effects in biologic systems.
From available evidence, we cannot yet answer the question as to whether micronutrient supplements actually improve health or decrease risk of disease where food cannot. In addition, whilst there are areas of health promise for some antioxidants presently available, there are conflicting data in relation to their adverse effects. For example, favourable effects of vitamin E have been observed in relation to Alzheimer's disease and prostate cancer, but the use of high doses of vitamin E is also associated with increased risk of mortality from some cancers, possibly fatal as opposed to non-fatal myocardial infarction, and haemorrhagic stroke. Beta carotene supplements, whether on account of the isomers used or because they have been used in isolation, have increased the incidence of tumours. they should no longer be used. Another area of concern about supplements is how much suppression of oxidation may be compatible with good health, as toxic free radicals are required for defence mechanisms.[20]

1.15 Antioxidants in clinical practice
High intakes of antioxidant nutrients from food sources appear to offer some health advantages. In addition, a diet high in fruit and vegetables often means a lower intake of fat and a higher intake of fibre, which may also protect against many diseases. Vitamin and mineral supplements do not necessarily make up for 'poor food habits' or 'unhealthy lifestyle practices'. It is advisable to eat a wide variety of cereals, fruit and vegetables in reasonable amounts rather than rely on supplementation with a few antioxidants.
Claims that antioxidant supplements have a therapeutic benefit are scientifically unjustified at present. Antioxidant activity determined in vitro may not be relevant in vivo; antioxidant nutrients have many functions, and may act through other mechanisms rather than as antioxidants. Prevention of disease through dietary supplementation may be a worthwhile objective, but dose response data are required to evaluate pharmacologic and toxicologic effects. The promotion of antioxidants as therapeuticagents is inappropriate when their efficacy is unproven and their toxicology uncertain.
It is much more realistic to envisage claims that a wide variety of plant- derived food might be protective against excess oxidant activity whilst retaining the required level of such activity for defence against infection.[21]

1.16 ANTIOXIDANTS INHUMAN HEALTHANDDISEASE
Free radical damage to biological systems have been implicated in a large number of aging-associated diseases, from eye disorders such as cataracts and retrolental fibroplasias to cardio vascular disease and diabetes. Since there is such amyriad of aging-associated diseases, this review will limit its focus to cardiovascular disease and neuro degenerative diseases and the effect of antioxidant therapy.

1.16.1. Cardiovascular disease
Since cardiovascular disease is the primary cause of death in the USA, it is of chief importance to understand the etiology of atherosclerosis. Atherosclerosisis a disease of the arteries in which the innermost parts of the vessels, the intima, thicken. One of the primary types of thickening consists of fatty, slightly raised, narrow, yellow streaks. These streaks are rich in foam cells, which are distorted cells with a high lipid concentration that come from endogenous smooth muscle cells and from macrophages. These fatty streaks are likely the precursors of fibrous plaques, which have the effect of obstructing the arterial lumina. These plaques are also composed of various cellular debris,

cholesterol crystals, and lipid deposits. They cause disease by limiting blood flow to the body organs. Heart attacks (myocardial infarction) and strokes (cerebral ischaemia) result when an arterial lumenis totally occluded, often with a thrombus forming at the plaquesite. What role does cholesterol play in developing atherosclerosis? The leading theory holds that the vascular endothelium produces lesions when damaged by mechanical and chemical reactions. Localized injured areas have increased permeability, resulting in a localized increase in the sub endothelial space in concentrations of serum components such as LDL. With increased serum concentrations, more monocytes will attach to the subendothelial space, infiltrate, and develop into macrophages. Activated monocytes and macrophages cause damage to the endothelial cells by secreting O_2^-, H_2O_2 ,HOCl, NO^- andhydrolytic enzymes. Other damaging compounds are continuously being produced in the significantly improved by vitaminC. Interestingly, this effect was reversed by NG monomethyl-l-arginine, a potent inhibitor of nitric oxide synthase.

Another example concerns the drug probucol; it is used clinically tol ower blood cholesterol levels, but it is also a potent antioxidant. In a recent experi-ment, atheroscleroticles ions in Watanabe heritable hyperlipidemic rabbits regressed when treated with probucol, and this anti-atherogenic effect was far greater than expected merely from its cholesterol-lowering capability. Thus, the researchers suggested that its antioxidant properties contributed to its anti-atherogenic effect. A study devoted to determining if probucol would decrease vascular O_2 production confirmed that it does decrease vascular superoxide production in cholesterol-fed rabbits. Probucol treatment, incholesterol fed rabbits, normalized both O_{2-} production and endothelium-dependent relaxation to acetylcholine. Thus, the researchers suggested that probucol may prevent O_{2-} induced in activation of endothelium-derived NO. Another recent study done on antioxidants and their anti-atherogenic effects concerns the Chinese herb, Salviamiltiorrhiza Bunge (SMB), which is widely used for the treatment of atherosclerosis-related disorders. The water- soluble polyphenolicantioxidant,SalvianolicacidB(Sal B),wasisolatedfromtheroots ofthisplantandwasfoundtoscavenge 1,1-diphenyl-2-picrylhydrazylradicals andinhibitLDLoxidationmoreeffectivelythanpr obucol.Inanexperiment usingrabbitsfedonahighcholesteroldiet,endoth elialdamageat6weekswas foundtobereducedby53%intheSMBgroup.``SM Btreatmentalsoreduced theatherosclerotic areaintheabdominalaortasi gnificantlyby56%andcholes-teroldepositioninthethoracicaorta by50%".

Lastly,veryexcitingresultshavebeenobtained inrabbitsundergoingdietsupplementationwith flaxseedoil.Flaxseedisarichsourceofomega-3fattyacids andlignansandisknowntohaveantioxidanteffec tssinceitsuppressestheproductionofoxygenfre eradicalsbyPMNLsandmonocytes.Flaxseedals o suppressesinterleukin-1(IL-1),tumornecrosisfactor(TNF),leukotrieneB4 (LTB4),andplateletactivatingfactor(PAF).Since thesecompoundsareknown tostimulatePMNLstoproduceoxygenfreeradica ls,suppressingtheirproduction willalsodecreaselevelsoffreeradicals.Inawell-designedexperiment,flax-seed(typeIflaxseed)reducedhypercholesterol emicatherosclerosisby46% with-outsignificantlyloweringserumlipids.Inafollo w-upstudyontypeII flaxseed,itwasfoundthatthistypeofflaxseedred uced thedevelopment of atherosclerotic plaque by 69%.[22]

1.16.2.Neurodegenerativediseases

Sincethe brainisoneof the body'smostmetabol ically activeorgansconsumingoxygenatarateof 35ml/min/kg,comparedtotheheart'soxygenco nsumption rateof59ml/min/kg,itishighly susceptibletoda magefromfreeradical processes. The brainisalso uniqueinthatit gen eratesoxidantsin waysthatareforeigntootherbo dysystems.Forexample,metabolism ofexcitatoryaminoacids andneurotransmittersgeneratesreactiveoxyge nspecies,andtheconstantuseof oxygen byneuralmitochondriaresultsinhighsuperoxid e levels

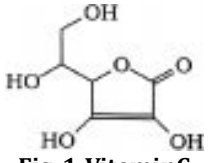

Fig. 1:VitaminC

discussed earlier inthis paper. Recently, it hasbeen found that endogenous guanidino compounds like guanidinoglutaric acid can form highly reactive species such as superoxide and hydroxyl radical in aqueous solution.

The brain also appears to be particularly vulnerable to free radical damage because it lacks or has localized many of the antioxidant enzymes. For example, SOD is localized primarily in neurons, and GSH and GPX are localized in astrocytes. Alsothere appears to be very little activity of Cat in the brain.

Thehypothesisthatage-associated,chronicneurodegenerativediseaseslike Alzheimer's disease and Parkinson's disease arerelatedtooxidativestressis well-supportedbymanyfindings.First,therearenumerousreportsintheliteraturethatpatientswithneurodegenerativediseaseshavedamagedmitochondria coupledwithhighlevelsofoxidativedamage. Second,thereare reportsthatinthecerebrospinalfluid there is free, nonproteinbound. The brain normally has particularly high levels of iron in the globuspallidus and substantianigra, which is believed to be due to the necessity of iron for the correct binding of neurotransmitters and receptors. However, in Alzheimer's disease there are increased neuronal iron concentrations due to increased neurofibrillary tangles. In Parkinson's disease, it was discovered that the total iron content of the substantianigra was 77% higher than other brain region These findings are significant because nonprotein bound Fe3+,through the Haber-Weiss reaction, can be reduced by superoxide to become Fe^{2+}.Fe^{2+}can then, in turn, react with hydrogen peroxide to form the highly reactive hydroxyl radical.These effects are believed to contribute to high levels of oxidative stress and the concomitant rapid peroxidation of highly abundant un-saturated brain lipids .[23]

1.16.3.Alzheimer'sdisease

The role reactiveoxygenspeciesplay intheetiologyof Alzheimer'sdisease iscurrentlyanareaofintenseresearch.Itiswell-knownthatAlzheimer'sdiseaseis clinicallyassociatedwiththedevelopmentofamyloidplaques.Itisbelievedthat theseplaquesarecausedbytheinproperfoldingandprocessingofamyloidb-precursorprotein(AbPP).AggregationofAbPP mayinvolvefreeradicals, anditwasfoundthatAbPP canitselfgeneratepeptidylfreeradicals. These findingsaresupportedbythediscoverythatthesynthetic,invitroformationof amyloidplaquesfromAbPPcanbeacceleratedby the presence of oxygen; in fact, these amyloidplaques themselves appear to stimulate the production of reactive oxygens pecies.

At this point intime, much work is being done in developing antioxidant therapies for Alzheimer's disease. For example, researchers discovered that melatonin can protect neurons against AbPP toxicity and resistance to proteolysis. It was found that melatonin interacts with Ab-40 and Ab-42 to inhibit the formation of b-sheets and amyloidfibrils, the formation of which determines the toxicity and proteolytic resistance of AbPP. Researchers in Germany are examining the protective effects of vitamin E and estrogento limit oxidative stress in the long run. Also under investigation in idebenone, asynthetic free radical scavenger which traps electrons, lazaroids (21-aminosteroids), pyrrol pyrimidines, NO_ blockers, selegiline, and a-lipoic acid.

There is also evidence that extracts of ginkgo biloba are useful in the treatment of Alzheimer's disease. It has previously been demonstrated that extracts of gink-gobiloba confer give protection for neuronal cells against conditions of impaired oxidative phosphorylation. This herb also canactasa free radical scavenger, and it has the capability to prevent peroxidation of lipid membranes by oxidants. Recently, researchers in Germany conducted a clinical, double-blind, placebo-controlled study on 20 out patients give normal doses of 240mg/day of a specially manufactured ginkgobiloba extract, EGb761,forthreemonths.The purposewastodetermineiftheextractcouldstabilizecognitive performanceor delaytheprogressionofAlzheimer'srelateddementia.Usingvariouspsycho-metric tests, therewasfoundtobeastatisticallysignificantimprovementbetween thebaseline valuesandfinalvaluesforthese testsamongthe group beingactively treatedwithEGb761.AnotherstudyonEGb761achievedsimilarresults: inthisyear-longstudy,onegroupofAlzheimerpatientstook40mgofEGb761 threetimesaday,whileasecondgroupofpatients receivedaplacebo.Onthe ``GeriatricEvaluationbyRelative'sRatingInstrument''test,theginkgogroup improveddailylivingandsocialbehaviorby37%, comparedto23%bythosein theplacebogroup.Also,overthecourseofthe study, thecondition of only19% oftheginkgogrouppatientsdeteriorated,compared to 40%ofthe placebogroup. Inconclusion,theresearcherssummarizedthat

EGb761couldstabilizeand,in somecases,improvethecognitiveperformance andthesocialfunctioningof Alzheimerpatientsfor6monthsto1year.[24]

1.16.4. Parkinson's disease

Dopaminergicneuronsareuniquelyvulnerablet odamageand disease. Theirloss inhumansisassociatedwithdiseasesoftheaged, mostnotably,Parkinson'sdisease.Parkinson'sd iseaseinvolvesthelossofdopaminergicneurons, especially inthemidbrainareacalledthesubstantianigra.O xidativestressisknownto playamajorroleinthedestructionoftheseneuro ns.Forexample,sinceFe3+is increasedinthesubstantianigraandhydrogenp eroxideisalsoproduced duringdopaminemetabolisminthedopaminerg icneurons,thereisthe possibilityofFe3+catalyzedproductionofhydro xylradicalswhichwillresultin significantdamagetotheseneurons.Hydroxylra dical production is also increasedwhenthemitochondrialrespiratoryc haindysfunctions,ashasbeen foundindiversetissuesofParkinson'spatients.O therfreeradicalsaregeneratedwhendopamine undergoesautoxidationorisenzymaticallyoxidi zedby monoamide oxidase. Hydrogen peroxide produced in such reactions could then react with the free iron to form hydroxyl radicals Which react to damage biomolecules and could lead to the loss of dopaminergic neurons. Another source of neurotoxicity may be the release of copper ions in the presence of l-DOPA; it has been demonstrated that l-DOPA and dopamine can damage DNA by oxidizing it in the presence of copper ions and hydrogen peroxide. Furthermore, research has shown that there is an increased production of superoxide in the mitochondria of the substantianigra along with increased levels of activity of SOD . In fact, the severity of Parkinson's symptoms has been correlated with the degree to which malondialdehyde and hydroperoxides are increased and GSH levels are decreased.

Moreover, many studies have been done on the toxic metabolite 1-methyl-phenyl pyridium (MPP+) of the neurotoxin 1-methyl-4-phenyl-1,2,3,6tetrahydropyridine (MPTP). In monkeys and other animal models, MPP+ induced Parkinson's disease-like symptoms and caused neuronal loss in the substantianigra. It was also found that MPP+ inhibits the mitochondria's respiratory chain complex, This intensively researched animal model for Parkinson's disease is being actively used to examine the role oxidants play in MPTP toxicity.

The loss of the dopaminergic neurons results in decreased dopamine production in various regions of the brain, including the cortex, nucleus accumbens, striatum, and thalamus . Since these areas are involved in controlling voluntary movement, loss of dopamine results in improper signaling which causes the characteristic clinical symptoms: jerky movements, trembling of the hands and lips, muscle rigidity, body tremors, a shuffling gait, and eventually loss of the ability to control voluntary movements.

Many of the antioxidant therapies employed in the treatment of Alzheimer's disease are also being used to treat Parkinson's disease, e.g., melatonin. Low doses of 6-hydroxydopamine (6-OHDA) are known to induce apoptosis of undifferentiated and differentiated PC12 cells by generating free radicals, and this sys-tem has been proposed as an experimental model for Parkinson's disease. Significantly, melatonin was found to prevent apoptosis by 6-OHDA in neuronal cells, perhaps by scavenging free radicals and increasing the mRNA levels and the activity of antioxidant enzymes. Melatonin and its precursor, N-acetyl-serotonin (normelatonin), have also been found to protect human neuroblastoma SK-N-MC cells and primary cerebellar granular neurons against oxidative stress .Another study investigated the effects of melatonin on rescuing dying cells (100% tau+ neurons), including tyrosine hydroxylase immunopositive dopamine neurons.

Apotosis was prevented in these cell lines, and this effect was dose and time dependent and was mimicked by other antioxidants such as 2-iodomelato-nin and vitamin E. Melatonin also prevented the usual 50% loss of dopamine neurons caused by neurotoxic injury induced by 1-methyl-4-phenylpyridine. These remarkable results indicate that melatonin possesses a tremendous ability to rescue neurons from cell death

Studies using other experimental models of Parkinson's disease found that inhibition of monoamine oxidase B (MAO-B), the enzyme responsible for the oxidation of dopamine, reduces oxidative stress on the dopaminergic neurons.

can prevent neuronal degeneration. In mutant mice lacking the gene for MAO-B, researchers found that these mice were resistant to the neurodegenerative effects of MPTP. Moreover, in a double-blind study using selegiline (deprenyl) to inhibit MAO-B, it was established that selegiline can delay the need

to be treated with l-DOPA by 10 months. More recent studies have confirmed this antioxidant effect. For example, a study examining the effect of tocopherol and deprenyl on the progression of Parkinson's disease discovered that deprenyl (10 mg per day) but not tocopherol (2000 IU per day) delays the onset of disability associated with early Parkinson's disease.

Also under much investigation are thiol antioxidants, such as GSH, N-acetyl-cysteine (NAC), and dithiothreitol (DTT). These antioxidants and metal chelators, along with vitamin C, have been shown to prevent dopamine autooxidation, they also act to inhibit dopamine-induced apoptosis Other potential anti-oxidant treatments include a lipoic acid , lazaroids , bromocriptine, and estrogen. Recently, estrogen replacement therapy has been shown to be protective against the development of Parkinson's disease-associated dementia.[25]

CONCLUSION

On the basis of above study following conclusions can be drawn

1. Free radicals are very harmful to human health and can cause several degenerative diseases like diabetes, cancer, atherosclerosis, hypertension etc
2. Various kinds of antioxidants particularly from natural sources such as enzymes, tocopherol, carotenoids, ascorbic acid, polyphenols etc. inhibits the cellular damage mainly through free radical scavenging property.[26]

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