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Review Article

Significance of antioxidants in human health

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Abstract: From the last few years, there has been a great attention towards the field of free radical chemistry for health point of view. Free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous and exogenous systems. There should be balance between free radicals and antioxidants for proper physiological function of human body as their imbalance causes numerous diseases. Antioxidants are essential and important for plants and animals' sustenance. They are substances that protect cells from the damage caused by unstable molecules known as free radicals. This article presents sources, types, mechanism of action and damaged caused by free radicals in human body. To neutralize the effect of free radicals, the role of antioxidants, their classification, types and mode of action has been discussed.
Keywords: antioxidants, free radicals, human, health

INTRODUCTION

One of the main driving forces, which help in sustaining human life, is the biochemical reaction, which takes place within the cell organelles [1]. The laws of nature dictate that one goes from childhood to adulthood and finally enters a frail condition that leads to death [2]. Ageing, which is a common process of life cycle of virtually all multi-cellular organisms, will lead to an increasingly elderly population in developed countries due to low birth rates and increasing life expectancy [3] and this stage in human life is characterized by diseases of cardiovascular, brain and immune system, which translate into high social cost [1]. In recent past, there is an increasing awareness among people in prevention of diseases, especially caused by the free radicals, which are continuously produced by the body's normal use of oxygen [4], which is an indispensable element of life [5], as its oxidative property plays a vital role in various biological phenomena, and oxygen being essential for life can also aggravate the damage within the cell by oxidative events [6]. Human body derives energy from the utilization of nutrients and oxygen as a fuel [7] and also utilizes oxygen to help immune system, destroy foreign substances and combat diseases but it is ironic that oxygen under certain situations has deleterious effects on the human body [8]. Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as reactive oxygen species (ROS) as well as reactive nitrogen species (RNS), which have a tendency to donate oxygen to other substances [9]. These two species play a dual role, i.e., toxic and beneficial, in human body, thus, maintaining balance between these two antagonistics in the body is an important aspect of life [10]. At low or moderate levels, these reactive species exert beneficial effects on cellular responses and immune function, while they produce oxidative stress, which damages the cell function and structures at high concentration [10, 11]. This article deals with the synthesis mechanism of these reactive species (free radicals), diseases caused by them and antioxidants.

MECHANISM OF FREE RADICALS FORMATION

Free radicals can be defined as reactive chemical species with one or more unpaired electron in its outer shell [12-17]. They are formed due to homolytic cleavage of a chemical bond so that each atom may have one of paired electrons by cleavage of a radical to give another radical and also via redox reactions [12, 13].

$$A\text{-}B \longrightarrow A^{\cdot} + B^{\cdot}$$

The radical might donate or accept an electron from other molecules in order to pair or it might simply

join to the molecule, therefore, behaving as oxidant or reductant [18]. When these radicals give or take an electron or add to the anion to become a radical, the reactions generally continue as a chain reaction, in which, one radical begets another [19]. They are highly unstable and active towards chemical reactions with other molecules [2] and are derived from three elements viz., oxygen, nitrogen and sulfur, and thus, creating reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS). These include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), peroxyl ($ROO^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$). hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3) and lipid peroxide (LOOH) are not the free radicals though they are generally known as oxidants and they can easily lead to free radical reactions in living organisms [20]. Majority of them are highly reactive species, thus, they are usually known as reactive oxygen species (ROS), which are capable to damage biological systems such as deoxyribonucleic acid (DNA), proteins, carbohydrates and lipids [21]. 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.

SOURCES OF FREE RADICALS

Free radicals can be produced from two principle sources, i.e., endogenous and exogenous (Fig.1). These reactive species are produced in animals and humans under physiologic and pathologic conditions [15, 22, 23, 24].

- Endogenous sources include those that are formed within the cell but released into the surrounding area. These can be enzymatic reactions, which serve as a source of free radicals. Intra-cellular free radicals are formed as a result of auto-oxidation, due to the activity of certain enzymes such as oxidases, lipoxygenases, cyclo-oxygenases, dehydrogenases and peroxidases, and electron transfer from metals such as iron to oxygen-containing molecules can also initiate free radical reactions [25]. A wide range of free radical molecular species is endogenous. The singlet oxygen is not a free radical but it is a reactive oxygen species (ROS) and capable of causing tissue damage [26, 27].
- Exogenous sources include non-enzymatic reactions of the oxygen with organic compounds. They also arise in reactions initiated by ionizing radiations. Some other external sources of free radicals production are irradiation, chemical pollutants and some medications, including cancer chemotherapeutic agents. These exogenous sources resulting from ionizing radiation play an important role in the production of free radicals. The energy transferred from ionizing particles into water ionizes the water molecule and produces water ions [26, 27, 28].

The amount of free radical production is determined by the balance of many factors, and the reactive oxygen species are produced both through endogenous and exogenous sources. Sometimes, the physiological factors are also responsible for the production of free radicals like stress, emotion, etc.

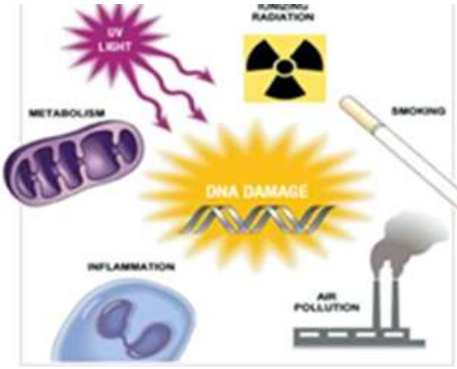

Fig. 1: Sources of free radical

TYPES OF FREE RADICALS

Though a number of free radicals are formed in the body but the most important radicals are derivatives of oxygen better known as reactive oxygen species [18], which include:

- **Hydroxyl radical (OH^-):** It is the neutral form of hydroxide ions (HO^-), which are highly reactive, short-lived and most damaging radicals in the body. They can damage nearly all types of macromolecule like carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation) and amino acids, e.g., conversion of phenylalanine (Phe) to m-tyrosine (Try) and o-tyrosine [29] Fig.2. Hydroxyl radicals are very short lived in vivo of approximately 10^{-9} seconds and highly reactive [30], which makes it a very dangerous compound to the organism [29, 31].

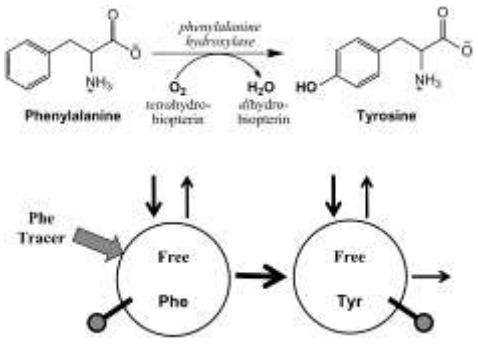

Fig.2: Conversion of phenylalanine to tryosin

- **Superoxide radical ($O_2^{\cdot-}$):** It is also known by its obsolete name, hyperoxide. It is biologically quite toxic and is deployed by the immune system to kill invading microorganisms. In phagocytes, super oxides are produced in large quantity by the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase for use in oxygen-dependent killing mechanisms of invading pathogens. It is formed when oxygen acquires an additional electron, leaving the molecule with only one unpaired electron.

- **Hydrogen peroxide (H_2O_2):** It is produced in vivo by many reactions. It can be changed to highly harmful hydroxyl radical or catalyzed and excreted safely as water. Glutathione peroxidase is essential for the conversion of glutathione to oxidized glutathione, during which, hydrogen peroxide is converted into water [32]. If hydrogen peroxide is not converted into water, singlet oxygen ($^1O_2$) is formed.

- **Singlet oxygen:** It is not a free radical though it is molecular oxygen (O_2) with higher energy than the ground state triplet oxygen. It can be produced by various methods but usually it is generated with a photosensitizer pigment. It has different chemical properties than triplet oxygen, including absorbing and emitting light at different wavelengths [33].

- **Triplet oxygen ($^3O_2$):** Triplet oxygen is the ground state of the oxygen molecule. The electronic configuration of the molecule has two unpaired electrons occupying the two degenerate molecular orbitals and classified as anti-bonding molecular orbitals [33]. The unpaired electrons in degenerate orbitals can have the same spin. Due to this, the total spin (S) of the molecule is one and it is known as a triplet configuration since the spin has three possible alignments in an external magnetic field.

MEASUREMENT OF FREE RADICALS

With increasing interest in the role of free radicals, there is need for techniques to measure free radicals. Several problems arise when considering measurement of free radicals [18] due to ultra-short half-life of these radicals (usually measured in microseconds). They can be measured by using electron spin resonance and spin trapping methods as given below:

- **Electron spin resonance (ESR):** This method is very sophisticated and able to trap the shortest-lived free radical. Exogenous compounds with a high affinity for free radicals are utilized in the spin techniques. The compounds and free radical together form a stable entity that can easily be measured. This indirect approach has been termed fingerprinting [34]. However, this method is not 100% accurate, as it has poor sensitivity and can skew the results [35].

- **Spin trapping:** When the half-life of radicals is too short to detect with ESR, the compounds known as spin traps are used to react covalently with the radical products and form more stable product that will also have paramagnetic resonance spectra detectable by ESR spectroscopy. The use of radical-addition reactions to detect short-lived radicals was first proposed by E.G. Janzen in 1965 [36].

HARMFUL EFFECTS OF FREE RADICALS

Free radicals are highly reactive if not inactivated. Their reactive nature is capable of damaging almost all types of biomolecules including proteins, carbohydrates, lipids and nucleic acids. Free radicals are molecules, usually of oxygen, consisting of two atoms of oxygen. On combination, they balance each other. If these two atoms are separated, they form two free radicals, and this loss makes them unstable. As soon as these free radicals are created, they are checked by enzymes in the body, which are known as antioxidant enzymes, or nutrients in food that are known as antioxidant nutrients like vitamin C, vitamin E, beta-carotene and bioflavonoids [37]. Trouble arises if the generation of free radicals is much more and enzymes are unable to handle it. These free radicals can destruct cell membranes, proteins and lipoproteins by a process known as lipid peroxidation. Proteins may also be damaged by ROS/NOS, leading to structural changes and loss of enzyme activity [38]. They can attack DNA, leading to dysfunction, mutation and cancer. The body has several mechanisms to counteract these attacks by using DNA repair enzymes and/or antioxidants [39]. Jointly, it ultimately results in arthritis, emphysema in lungs and bronchitis, atherosclerosis in the blood vessels and ulcer in stomach, i.e., in each part of body these radicals can produce damage. They are also responsible for ageing [8].

ANTIOXIDANTS

Antioxidants are fabricated or natural substances, which may prevent or delay some types of cell damage [40]. According to literature, these are substances those when present in low concentration compared to those of the oxidisable substrates significantly delay or inhibit the oxidation of that substance [41]. Diverse free radical scavenging antioxidants are found in dietary sources like fruits,

vegetables, tea, etc. [42]. They act as an inhibitor of the oxidation process, even at relatively small concentration and thus have diverse physiological role in the human body. Antioxidants of plant origin act as radical scavengers and help in converting the radicals to less reactive species. Antioxidants prevent cell and tissue damage. Cells have the defense against excessive free radicals by their preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses [43]. Antioxidants came to public attention in the 1990s, when scientists began to understand that free radical damage involved in the early stages of artery-clogging atherosclerosis and may contribute to cancer, vision loss and a host of other chronic conditions. Some studies showed that people with low intakes of antioxidant-rich fruits and vegetables are at greater risk for developing these chronic conditions than the people who ate plenty of these fruits and vegetables.

CLASSIFICATION OF ANTIOXIDANTS

Antioxidants are broadly grouped into two categories, i.e., (A) natural antioxidants and (B) synthetic antioxidants

A. Natural antioxidants: They are the chain breaking antioxidants, which react with lipid radicals and convert them into more stable products. They include retinoids (vitamin A), bioflavonoids (citrin), polyphenols (hydroxytyrosol), tocopherols (vitamin E) and ascorbic acid (vitamin C). They play a significant role in the prevention of cancer, heart disease, ageing and immune deficiency diseases. Antioxidants mainly have phenolic structures including antioxidants minerals, antioxidants vitamins and phytochemicals [44]. The natural antioxidants are either endogenous or exogenous in nature:

- **Endogenous antioxidants:** These are produced by the body and classified as (i) enzymatic and (ii) non-enzymatic.

(i) Enzymatic antioxidants: These antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reducates (GRx) as reported by Bouayed and Bohn [45]. They are beneficial for human beings as they break down and remove the free radicals from the body. They can wash out dangerous oxidative products by converting them into hydrogen peroxide and then into water. This is done through a multi-step process that requires a number of trace metal cofactors, such as zinc, copper, manganese and iron. Enzymatic antioxidants cannot be found in supplements but they are produced in human body [46].

(ii) Non-enzymatic antioxidants: These antioxidants are beneficial for human beings as they interrupt free radical chain reactions and further divide into metabolic antioxidants and nutrient antioxidants. They are of five types, i.e., superoxide dismutase (SOD), alpha-lipoic acid (ALA), catalase and coenzyme Q 10 (CoQ10), glutathione peroxidase (GPX). Among these, superoxide dismutase, catalase and glutathione peroxidase are the most important since the body can produce most of them when certain free radicals are present [47]. Together, these three antioxidants can convert free radicals into oxygen and water, neutralizing them.

a. Superoxide dismutases (SOD): These are the enzymes that alternately catalyze the dismutation (or partitioning) of superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). It repairs cells and reduces the cellular damage caused by the most common free radicals in the body called superoxide, which also acts as an anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes.

b. Catalase: It is a common enzyme produced by aerobic organisms ranging from bacteria to man. It consists of tetramer of four polypeptide chains, each having over 500 amino acids [48]. It disintegrates the hydrogen peroxide into water and oxygen [49] due to the presence of four porphyrin heme (iron) groups, which allow the enzyme to react with hydrogen peroxide.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

c. Alpha-lipoic acid (ALA): It is an antioxidant, which protects the body cells from damage and also restores vitamin levels such as vitamin E and vitamin C used in memory loss, chronic fatigue syndrome (CFS), HIV/AIDS, cancer, liver disease, diseases of heart and blood vessels and Lyme disease [50]. It is a naturally occurring compound produced in the body and synthesized by both plants and animals.

d. Glutathione (master antioxidant): An intracellular tripeptide having unique ability of maximizing the performance of all the other antioxidants, including vitamins C and E, coenzyme Q10, alpha-lipoic acid, etc. is found in every single cell of human body. It can repair the damage done to DNA, killing cytotoxic T-cells. It is the most abundant and important endogenous antioxidant [51].

e. Coenzyme Q_{10} (ubiquinone, ubidecarenone, or coenzyme Q): It is a 1, 4-benzoquinone. Q refers to quinone and the 10 refers to the number of isoprenyl chemical subunits found present in its tail. It is similar to vitamin and found throughout the body, especially in heart, liver, kidney and

pancreas. It is found in small amount in meat, seafood, soya oil, sardines, mackerel and peanuts.

- **Exogenous antioxidants:** They enter the human body through diet, or supplements with antioxidant formulations and co-factors, i.e., copper, zinc, manganese, iron and selenium. The consumption of exogenous antioxidants makes the immune system strong and helps the endogens in combating diseases [52].

B. Synthetic antioxidants: These are synthetic chemicals approved by Food and Drug Administration for addition to foodstuffs and are more potent antioxidants than the natural ones [53]. Although they act as food preservatives and preclude undesirable decaying and deterioration but does not rule out the detrimental effects of long-term synthetic chemical abuse [54, 55]. They were first introduced into packaged food items in 1940s. From the beginning, they were chiefly added to edible fats and fat-containing foodstuffs because of their ability to prevent food from rancidity and developing unpleasant odour. They have the phenolic compounds whose function is to capture the free radicals and stop the chain reactions [44]. The common phenolic synthetic antioxidants are as follows:

i. **Butylated hydroxyl anisole (BHA):** It is an antioxidant consisting of a mixture of two isomeric organic compounds, i.e., 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole and used as a preservative in food since it prevents rancidification of food, which creates objectionable odour in packed food, animal feed, cosmetics, and rubber and petroleum products [56].

ii. **Butylated hydroxyl toluene (BHT):** It is also known as dibutyl hydroxyl toluene, which is a lipophilic derivative organic compound of phenol with useful antioxidant properties and used to prevent the oxidative rancidity of fats, food odour, colour, flavor, and sometimes, it is added directly to cereals and other food articles containing fats and oils, however, the evidences show that some persons may have difficulty in metabolizing the BHT, resulting in health and behaviour changes [57].

iii. **Propyl gallate (PG):** It is propyl 3, 4, 5-trihydroxybenzoate and an ester formed by the condensation of gallic acid and propane. Being an antioxidant, it protects the food, cosmetics, hair products, adhesives and lubricants from oxidation.

iv. **Tertiary butyl hydroquinone (TBHQ):** It is a derivative of hydroquinone, a highly effective antioxidant found in many foodstuffs such as snack crackers, noodles, fast and frozen food and also in branded pet food, cosmetic, baby skincare products, varnish, lacquers and resins. It is available in combination with other preservatives such as BHA, BHT, or PG. The Food and Drug Administration (FDA) allows up to 0.02% of the total oils in food to be TBHQ [58].

v. **Nordihydro guaretic acid (NDGA):** It is an antioxidant 5-LO (5-lipoxygenase) - a lignin inhibitor found in creosote bush (Larrea tridentate). Earlier, it was used to preserve natural fibers, but later, its use was banned after reports of toxicity during early 1960s [59], however, some reports show that this compound suppresses growth of cancerous cells in breast by inhibiting the function of key receptors.

Comparative properties of natural and synthetic antioxidant are given in Table 1.

Table 1: Comparative properties of natural and synthetic antioxidants

Natural antioxidants	Synthetic antioxidants
They have multiple health benefits.	They may cause adverse effects on human body.
They are readily acceptable by the body.	They require more effort in metabolism.
They can be used as pigment (anthocyanins) or flavoring spices.	Their property is only to stabilize oil.
They being of plant origin offer a variety of products.	They do not offer a variety of products.
They have antioxidant effects on human tissues.	They have no antioxidant effects on human tissues.
They may offer the quality of functional food (nutraceuticals) to oils.	They do not offer nutritional quality.
These should be consumed raw (benefits ceased by cooking heat).	Cooking heat does not affect their quality (recommended for frying).
They involve higher production cost.	They involve lower manufacturing cost.
These are still in research phase.	These are used on large industrial scale.
They could help in developing SMEs (smaller-scale production)	High productivity- in large enterprises

MECHANISM OF ACTION OF ANTIOXIDANTS

Free radicals such as superoxide, hydroxyl ions and nitric oxide, which contain an unpaired electron, can have a negative effect on cells, causing oxidative damage, which leads to cell death. These antioxidants in general act by the following routes [53]:

1. Chain breaking reaction, e.g., tocopherol, which acts in lipid phase to trap free radical
2. Reducing concentration of reactive oxygen species, e.g., glutathione
3. Scavenging initiating radicals, e.g., superoxide dismutase, which act in the lipid phase to trap superoxide free radicals

- Chelating transition metal catalyst- a group of compounds, which act by sequestration of transition metals that are well-established proxidants, in this way transferring lacto-ferrin and ferritin function to keep the iron induced oxidant stress under check and ceruloplasmin and albumin as copper sequestrants.

SOURCES OF ANTIOXIDANTS

The three primary types of antioxidant found in nature are phytochemicals, vitamins and enzymes but the antioxidants found in plants are most powerful since the plants are exposed to UV light throughout the day. Although antioxidants are synthesized within the human body but can be obtained from fruits and vegetables including nuts, grains, seeds, meats, poultry and fish in the form of vitamin E, C and beta-carotene [60]. The antioxidants defense within the cell are of two types, i.e., (i) fat soluble cellular membrane consisting of vitamin E, beta-carotene and coenzyme Q [61] and (ii) water soluble antioxidant scavengers present inside the cell consisting of vitamin C, glutathione peroxidase, superoxide, dismutase (SD) and catalase [62].

Vegetables are natural source of antioxidants, which not only act as modulators of signal molecules (O_2^- and H_2O_2) during maturation and stress conditions but also play an important role in the prevention of numerous degenerative diseases in humans. The most disorders commonly occurred in humans are due to the oxidative stress [63]. The antioxidant therapy has gained an immense importance in the treatment of numerous diseases. Velavan *et al.;* [64] reported to prevent oxidative damage caused by free radicals or reactive oxygen species, which may prevent the occurrence of disorders. The dietary components identified as antioxidants in vegetables are vitamins C and E, carotenoids, niacin, riboflavin and sulphur containing amino acids, which provide reducing thiol groups for proteins and reduced glutathione and have positive antioxidants properties. Phytochemicals like flavonoids and polyphenols also act as antioxidant.

NUTRIENTS BASED ANTIOXIDANTS

Vitamin A

Vitamin A is fat-soluble and found mainly in three forms, i.e., retinol (vitamin A_1), 3, 4-didehydroretinol (vitamin A_2) and 3-hydroxyretinol (vitamin A_3). It is good for healthy vision, skin, bones and other tissues present in the body. It often works as an antioxidant fighting against cell damage. The other functions include the formation and maintenance of teeth, bones, soft tissue, white blood cells, and immune system and mucus membranes. The food articles rich in vitamin A are yellow vegetables such as sweet potato, carrot, milk, cheese and animal sources such as liver, milk, butter, egg yolk and mozzarella dark green [65].

Beta-carotene (lycopene)

Carotenoids are the natural pigments, which are synthesized by plants and are responsible for bright yellow colour of various fruits and vegetables. There are dozens of carotenoids in foodstuffs eaten by human beings and most of these carotenoids have antioxidant activity [66]. They contain conjugated double bonds and their antioxidant activity arises due to their ability to delocalize unpaired electrons [67]. Carotenoids, especially the β-carotene, exhibit antioxidant properties at low or partial oxygen pressure but become pro-oxidants at high oxygen pressure, and similarly, at high carotenoids concentration, pro-oxidant behavior is displayed [68, 69]. Beta-carotene may act to inhibit changes in cellular modulation and cell cycle regulatory proteins and alterations in insulin like growth factor. These processes stop a cell from changing into a cancerous growth by preventing the process of oxidative DNA damage. It also stimulates increased level of T-helper lymphocytes, (NK) cell cytotoxicity (a natural form of cancer-killing cells) as well as macro-phage action. Its sources are green leafy vegetables and ripe yellow fruits and vegetables like papaya, mango, musk melon, pumpkin and carrot.

Vitamin C

Vitamin C (ascorbic acid) is a monosaccharide antioxidant found in both plants and animals. Most of the animals are able to produce ascorbic acid in their body and do not require its inclusion in their diet but humans are not able to synthesize ascorbic acid in their body because the enzymes required to synthesize ascorbic acid had lost during mutation of human evolution, therefore, the human body has to obtain it through diet [70]. In cells, it is maintained in its reduced form through a reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins [71]. Ascorbic acid- a reducing agent can reduce or neutralize ROS such as hydrogen peroxide [72]. In spite of this, it acts as a substrate for the antioxidant enzyme ascorbate peroxidase- a function, which is particularly important in stress resistance in plants [73]. Citrus fruits, orange, lemon, wet lime,

guava, goose berry and spouted pulses are rich source of vitamin C.

Vitamin E (Tocopherols)

Vitamin E is the most common naturally occurring antioxidant. It is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble [74]. It has a phytyl chain, which is attached to its chromanol nucleus [7]. Out of eight, α-tocopherol has been mainly studied because of its highest bioavailability to the body, preferentially absorbing and metabolizing form. It has been claimed that α-tocopherol form is the most important lipid-soluble antioxidant, which protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [75]. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized α-tocopheroxyl radicals, which can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol, or ubiquinol [76]. Its source is cereals, oil seeds and nuts.

Flavonoids

They are the group of polyphenolic compounds generally found in plants as glycosylated derivatives. This subgroup contains the anthocyanins, which are responsible for a wide range of colors in plants such as blue, scarlet, and orange. They are found in leaves, flowers, fruits, seeds, nuts, grains, spices, different medicinal plants, and beverages such as wine, tea, and beer [77- 80]. They can scavenge free radicals, particularly singlet oxygen due to their reversible oxidation-reduction properties [81]. They exhibit several biological effects such as antitumoural, anti-ischaemic, anti-allergic, anti-hepatotoxic, antiulcerative, and anti-inflammatory activities some dietary flavonoids are given in Table 2.

Table 2: Some dietary sources of flavonoids and phenolic acids

Flavonoids	Source
Catechin	Tea and red wine
Flavonones	Citrus fruits
Flavanols (e.g. Quercetin)	Onions, olives, tea, wine and apples
Anthocyanidins	Cherries, strawberries, grapes and coloured fruits
Caffeic acid	Grapes, wine, olives, coffee, apples, tomatoes, plums and cherries

Selenium

It is an essential trace element its deficiency has caused serious health effects in humans, such as Keshan disease [82]. It is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR) and iodothyronine deiodinases (IDD), there has been an increased interest in the study of other Se-containing proteins (selenoproteins) or enzymes (selenoenzymes) Tapiero *et al.;* 2003[83]. There are at least 30 selenoproteins that have been identified in mammals, and it has been estimated that humans have about 25 selenoproteins [84]. Plant foods like rice and wheat are the major dietary sources of selenium in most countries. The amount of selenium in soil, which varies by region, determines the amount of selenium in the foods grown in that soil. Meats, sea foods and cereals are the rich source of selenium.

PHYSIOLOGICAL EFFECTS OF FREE RADICALS IN HUMAN BEINGS

Free radicals are very damaging to human beings. In normal conditions, the free radicals that are produced within the body can effortlessly be neutralized by the antioxidants. The production of free radicals takes place while there is lack of equilibrium between pro-oxidant and the antioxidant defense system, due to which, the patho-physiological processes leading to cellular toxicity cause variety of disorders, including degenerative disorders of the central nervous system [85,86]. Free radicals and reactive oxygen species react with deoxyribonucleic acid, producing strand breakage, mutations leading to cancer and also interfere in the regulatory control of cell division, cell differentiation and cell death [82]. Besides, they can put negative effect on the performance of an athlete by slowing or halting muscle growth and by lowering aerobic capacity [87].

Oxidative stress

In 1986 Sies [88] elaborated a relationship between free radicals and disorders giving the concept of oxidative stress. In the body of a normal individual, the production of pro-oxidants in the form of ROS and RNS are efficiently checked by the antioxidant defense system of various levels to detoxify them readily [89, 15]. In human beings, oxidative damage and free radicals are strongly associated with a number of diseases including atherosclerosis [8], Alzheimer's disease [90], cancer [91], ocular disease [92], diabetes [93], rheumatoid arthritis [94] and motor neuron disease [95]. Defense against all of these diseases is dependent upon the adequacy of various antioxidants that are derived either directly or indirectly from the diet [96]. Amongst dietary supplements, vitamin E, vitamin C and beta-carotene are widely used. Out of these, vitamin E has promising effects in protecting against toxicity

induced by Vancomycin [97], Gentamycin [98] and Cisplatin [99]. Tempol and 2, 3-dihydroxy-benzoic acid can prevent Vancomycin-induced nephrotoxicity in rat [100] and antioxidants could ameliorate some of phenytoin adverse effects [101]. Several observational studies suggest that antioxidants should be able to prevent oxidative damage in humans who consume large amount of fruits and vegetables.

Cancer

The cause of cancer is usually due to damage of DNA. Sometimes, ROS and RNS such as super oxide anion, hydrogen peroxide, hydroxyl radical, nitric oxide and their biological metabolites play an important role in carcinogenesis [9]. ROS-induced DNA damage can take many forms, ranging from specifically oxidized purine and pyrimidine bases to DNA lesions such as strand breaks, sister chromatid exchanges (SCEs) and formation of micronuclei [102]. Various researchers have proposed the participation of free radicals in carcinogenesis, mutation and transformation. The major biological effect of radiation is induction of mutagenesis, which occurs mainly through damage of DNA by the $HO^{\bullet}$. These radicals and other chemical species are produced due to radiolysis or by direct radiation effect on DNA and add into the double bond of pyrimidine bases, which cause the removal of hydrogen from the sugar moiety because of this chain reaction in DNA starts, which causes cell mutagenesis.

Consumption of fruits, vegetables, herbs, or their phytochemical constituents in diet helps in cancer prevention [103-105]. Due to the antioxidant properties of some food articles, they protect cells from ROS-mediated DNA damage, which results in mutation and subsequent carcinogenesis [106]. They are also capable of interfering metabolic activation of chemical carcinogens, which cause regression of pre-malignant lesions or may inhibit their development into cancer [8]. There are many types of compound present in fruits and vegetables, which may potentially influence cancer risk. It is normally assumed that some of the antioxidants like vitamin E, vitamin C and beta-carotene may be responsible for lowering the rate of cancer. Vitamin E, an important antioxidant, plays a role in immune competence by increasing humoral antibody protection, resistance to bacterial infections, cell-mediated immunity, the T-lymphocytes tumor necrosis factor production, inhibition of mutagen formation, repair of membranes in DNA and blocking micro-cell line formation [107]. β- Carotene may be protective against cancer through its antioxidant function since oxidative products can cause genetic damage. It also has anti-carcinogenic effect by altering the liver metabolism effects of carcinogens [108]. Vitamin C is also helpful in preventing cancer possibly by blocking the formation of nitrosanimes, enhancing immune response and accelerating detoxification of liver enzymes [109].

Cardiovascular Diseases

Cardiovascular diseases are the major killers in the world. The terms universally used for different cardiovascular diseases are atherosclerosis, ischemic heart disease (IHD), hypertension, cardiomyopathies, cardiac hypertrophy, congestive heart failure [110], vascular thrombosis [111] cerebrovascular disease (CVD) and other related disease for example is myocardial infarction (MI). The ROS-induced oxidative stress in cardiac and vascular myocytes has been linked with cardiovascular tissue injury [112]. The major sources of oxidative stress in cardiovascular system involve (i) the enzymes xanthine oxidoreductase (XOR), (ii) NADPH oxidase- multisubunit membrane complexes, (iii) NOS, (iv) the mitochondrial cytochromes and (v) haemoglobin [113, 114]. Polyunsaturated fatty acids occur as a major part of low density lipoproteins (LDL) in the blood, and the oxidation of these lipid components in LDL plays a vital role in atherosclerosis [116]. The three most important cell types in the vessel wall, i.e., endothelial cells, smooth muscle cell and macrophage, can release free radical, which affect lipid peroxidation [117].

The consumption of antioxidant nutrients may mitigate the detrimental effects, and several basic research studies strongly suggest that progression of the atherosclerotic lesions can be delayed by intervention with anti-oxidants [8,118,119]. It has been advised that general population consumes a balanced diet with more emphasize on antioxidants [120]. Vitamin E is used to prevent oxidation of polyunsaturated fatty acids (PUFA) in the cell membrane. Evidences show that vitamin E is more effective against cardiovascular disease (CVD) than beta-carotene or vitamin C.

Ageing

Ageing usually can be defined as a progressive decline in the efficiency of biochemical and physiological processes after the reproduction phase of life [1]. Many theories have been put forward to explain the phenomenon of ageing [121-125], but in 1956, Denham Harman [126] proposed the ageing concept of free radical theory and the role played by the free radicals in the process of ageing [126] and this theory gained universal acceptance because of his intense research in the field of free radicals role in biological systems. In general, theories describing the process of ageing are mainly of two types, i.e., damage-accumulation theories and genetic theories [126,127]. Mitochondrial ROS production and oxidative damage to mitochondrial DNA result in ageing [90].

Antioxidants are naturally occurring substances that may provide protection against the damaging effects of free radicals on cells in human body, including skin. Vitamin E (tocopherol)-an antioxidant,

which is present in the skin, is found in various foodstuffs, such as vegetables, seeds, nuts and meat [128]. It helps the skin look younger by boosting collagen production and in turn reducing the appearance of fine lines, wrinkles and age spots [129,130]. Vitamin C- an essential nutrient, which can be obtained from the citrus fruits and vegetables, works in two ways, i.e., as an antioxidant and as a booster of collagen formation both of which are important in preserving and maintaining skin's appearance youthful [131-133]. Lycopene responsible for red colour of fruits and vegetables is a potent antioxidant [134], helping in improving the skin texture since it promotes collagen production and reduces the DNA damage that leads to wrinkles [115].

CONCLUSION

Free radicals have been implicated in the large number of major diseases. They can adversely affect many important biological molecules due to which loss of form and function. Such kind of undesirable changes in human body can lead to development of diseased conditions and shortening the life span of human beings. Smoking and alcoholism is common problem all over the world. Due to excessive smoking level of many important antioxidants in the body decreases, this is detrimental to the health. Report has shown that proper intake of antioxidants can protect our body against the damage induced by free radicals acting at various levels. Dietary and other components of plants form major sources of antioxidants. The traditional Indian diet, spices and medicinal plants are rich sources of natural antioxidants. To cap it up, there is need for proper orientation on the necessity of proper intake of balance diet which will definitely supply the much needed antioxidants.

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