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Antioxidant Therapy: Potential and Limitations

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ABSTRACT:
Reactive oxygen species (ROS) are generated under severe abiotic and biotic stress conditions as well as during normal physiological processes. ROS damage DNA, lipids, proteins and other cellular components leading to a number of degenerative diseases. Antioxidants like vitamin E, vitamin C, carotenoids, flavonoids, anthocyanins, and many others are gaining importance because of their ability to reduce or impede the oxidative damage in the cell. As a result, antioxidants are being widely recommended for therapy of many diseases like cardiovascular diseases, cancer, neurodegenerative diseases, ageing, cataract formation, male infertility, cystic fibrosis and viral infections. Natural antioxidants have several advantages over synthetic antioxidants. On the other hand antioxidants when administered in higher amounts exert detrimental effects on health. The dose and the route of administration of antioxidants are important factors to be considered before taking in any exogenous supplements of antioxidants.

Key words: ROS, Oxidative stress, Cardiovascular diseases, Cancer, Ageing.

INTRODUCTION

Oxygen because of its biradical nature readily accepts unpaired electrons to give rise to a series of partially reduced species collectively known as Reactive Oxygen Species (ROS) [1]. ROS are involved in normal cellular functions such as immune responses, cell signaling and cell response to noxia, induction of mitogenic response, ovulation and fertilization. Apart from their role in normal cellular processes [2], ROS when generated in higher amounts can have deleterious effects. They can bring about disturbances in redox homeostasis and thereby produce oxidative stress. In addition, oxidative stress is also a secondary effect of many pathogenic infections, senescence and abiotic stresses like air pollution, oxidant forming herbicides such as paraquat dichlolride (methyl viologen), 1,1'dimethyl-4,4'-bipyridinium, heavy metals, drought, heat and cold stress, wounding, UV light and highly intense light condition that stimulate photo inhibition in plants. Oxidative damage to DNA, lipids, proteins and cholesterol is the major consequence of oxidative stress in the cell. In case of lipids, peroxyl radicals attack the PUFA side chain of membrane lipids triggering a chain of lipid peroxidation in the cell membrane. This disrupts the membrane integrity which in turn primes cell disruption and also affects the function of membrane bound proteins such as enzymes and receptors [3]. Similarly, proteins also suffer oxidative damage resulting in enzyme inactivity and structural deformities.

In order to minimize the damaging effects of ROS, aerobic organisms have evolved both enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase as well as non-enzymatic antioxidants such as glutathione, uric acid, bilirubin and cytochrome. The human body does not synthesize sufficient antioxidants to compensate with damaging effects of ROS and hence there is a requirement of dietary antioxidants [4]. Fruits and vegetables supply required exogenous antioxidants like vitamin C, vitamin E, ubiquinone, lycopene, cryptoxanthin, lutein and xeaxanthin. It is also noteworthy that higher amount of antioxidants may also interfere with the protective effects of ROS present at moderate amounts specifically for those having low levels of ROS[5]. This raises the question over the feasibility of therapeutic use of antioxidants. This review aims to evaluate the potential and limitations of antioxidant therapy.

NATURAL ANTIOXIDANTS

Living organisms have an effective antioxidant system as a defence against oxidative stress. These defence mechanisms include [6]– (1) suppression of generation of ROS, (2) scavenging ROS, (3) clearance, repairing and reconstitution of damage and (4) induction of antioxidant proteins and enzymes. However, these defense systems have antioxidants only enough to scavenge the ROS generated during normal physiological processes. The excess load of ROS generated under stress conditions create an imbalance in the redox equilibrium and is suggested as root cause of many diseases. In order to correct the perturbed homeostasis and prevent the onset, as well as treat the diseases prompted by oxidative stress the body needs to be enriched by exogenous antioxidants. Also, the criticism faced by commercial antioxidants due to the possible toxic effects has led to the search of antioxidants from natural sources [7]. Synthetic antioxidants like butylated hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) have been reported to cause liver swelling and influence liver enzyme activities. Plants are surfeited with mechanisms, both enzymatic and non-enzymatic, to combat increased ROS levels during stress conditions. Research in recent past has accumulated evidences that natural antioxidants from plant sources help in lowering - incidence of cardiovascular diseases, cancer, neurodegenerative diseases and acceleration of aging

process [8]. Several vegetables and fruits have been reported to possess antioxidant activity (Table 1).

OXIDATIVE STRESS AND ANTIOXIDANTS IN DIFFERENT DISEASES

The imbalance between the pro-oxidants and antioxidants in favour of pro-oxidants is called oxidative stress. Several biotic and abiotic factors play a vital role in producing oxidative stress. Oxygen is one of the primary requirements for living organisms. However 2% of electrons of the electron transport chain escape out of mitochondria and reacts with oxygen to form O_2. As a result mitochondria become the major source of reactive species producing approximately 2 kg of free radicals per year [9].During physical exercise the ATP requirement increases, this further increases production of free radicals in mitochondria. ROS are also produced by a family of membrane-bound enzymes such as NAD(P)H oxidase and certain soluble oxidases like xanthin oxidase[10].The phagocytic cells produce ROS in order to kill the foreign organisms. However, the ROS generated in this way can also harm the normal cells. Also, activated phagocytes show increased consumption of oxygen and produce oxidative burst. The process of phagocytosis can be said to be a major player in production of oxidative stress. Further, when the sequesteration of metal ions like iron and copper is incomplete, the free metal ions participate in catalyzing free radical damage. For example, in premature babies, where the transferrin protein is saturated, the free iron acts as a catalyst to free radical reactions. These factors either cumulatively or individually bring about oxidative damage to DNA, proteins and lipids.

CARDIOVASCULAR DISEASES

Several studies have revealed the role of oxidative stress in induction of many cardiovascular complications such as atherosclerosis, ischemic injuries, hypertension and congestive heart failure (Figure 1). One of the important factor that leads to atherosclerosis is the formation of LDL-ox and its deposition in arterial intima [15] leading to atherosclerotic lesions[16].

Traditional medicines used as a therapy to cardiovascular diseases are shown to have antioxidant properties. Several epidemiological studies have also suggested effect of antioxidant supplements towards decreasing risk of death from cardiovascular complications[17,18,19]. However, other constituents of diet and their concentrations along with type of oxidants they deal with may influence their role in combating oxidative stress and associated complications.

Many pharmaceutical drugs prescribed to cure diabetes mellitus and cardiovascular complications have been shown to have antioxidant activity along with their main stream pharmacological effect. For example, many of these are calcium channel blockers (CCBs) that mediate their effect of balancing intracellular Ca^{++} levels also behave as antioxidant due to their free radical scavenging capacity and protective effects towards SOD. These CCBs also reduce production of angiotensin-II and endothelin[24]; involved in worsening oxidative stress associated vascular complications. Some hypoglycemic drugs such as Gliclazide act directly as free radical scavenger, helping in reducing complications associated with diabetes mellitus[21]. Some other drugs having promising antioxidant activity are thiozoldines, HMG CoA reductase inhibitors (statins) and inhibitors of rennin- angiotensin system.

The observed action of vitamin C against cardiovascular diseases may be because of its protective effect on, nitric oxide oxidation, decreasing lipid peroxidation and mediating vasodilation in bronchial and coronary diseases. However, some evidences suggest the role of ascorbic acid as pro-oxidant when administered after paraquat[23]. Inconsistency has been observed in results of studies involving ascorbic acid supplementation for cardiovascular diseases demanding further investigation in this field. In one of the studies, vitamin E was found to preserve endothelial function by reducing vascular oxidative stress[24]. Also, it prevents activation of PKC, inhibiting migration and proliferation of vascular smooth muscle cells. Consumption of vitamin E rich foods such as nuts, margarine, and mayonnaise have been shown to be inversely associated with coronary mortality[26]. Similar results are obtained in a study involving post menopausal women suggesting a role of dietary vitamin E in combating oxidative stress [27]. Available evidences suggest that excess daily intake of viamin E affects platelet function and also interfere with vitamin K function and granulocyte response, perhaps because of its pro-oxidant action under certain circumstances.

Other antioxidants studied for their relation to cardiovascular diseases are α-lipoic acid, lycopene and folate. However, evidences report either no or negative role of folate, negative role of lycopene and positive role of α-lipoic acid. Similar effects are observed with vitamin A and carotenoids[28]. Hence, available data fails to recommend routine utilization of antioxidant vitamin supplements and other exogenous antioxidants for the prevention of coronary diseases, cardiovascular complications or stroke. However, dietary intake is recommended for its beneficial effect.

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Table 1: Antioxidant activity in certain fruits and vegetables

Sr.No	Botanical name	Common name	Part of the plant having antioxidant activity	Reference
1	*Camellia sinensis L.*	Tea	Leaves	[11]
2	*Mangifera indica L.*	Mango	Leaves	
3	*Allium sativum L.*	Garlic	Bulbs	
4	*Ocimum sanctum L.*	Tulsi	Leaves	
5	*Daucus carota*	Carrot	Flesh, leaves, peel	[12]
6	*Avena sativa*	Oat	Chaff	
7	*Fragaria ananassa,*	Strawberry	Fruit	
8	*Allium cepa*	Onion	Fruit	
9	*Solanum tuberosum,*	Potato	Peal	
10	*Citrus sinensis*	Orange	Fruit pulp	[13]
11	*Lycopersicum esculentum*	Tomato	Fruit	
12	*Malus pumila*	Apple	Fruit pulp	
13	*Musa sapientum*	Banana	Fruit	[14]

CANCER

Cancer has been a major disease all over the world. Free radicals have been reported for their role in etiology of cancer. Evidences from recent studies support the role of ROS in tumor initiation, promotion and progression[29], ROS are associated with mutations in mammalian cells, alterations in metabolic activity and hence transformation of normal cells into cancerous cells. One of the reasons for the generation of oxidative stress in the cell is the production of superoxide radicals in mitochondria during oxidative phosphorylation. These reactive species damage the mt-DNA or bring about mutations in it. These mutations affect the function of the encoded proteins and lead to the malfunction of mitochondrial respiratory chain, which further encourages the excessive production of ROS in the cell. Replication of this mutated DNA prior to its repair can root transversion mutation in DNA. Such mutations have been reported in genes whose dysfunction is involved in genesis of cancer[30]. The production of higher levels of ATP required by the mitochondria of the cancer cells, further adds to the ROS stress in the cancerous cell. ROS mediated DNA mutations may direct the activation of oncogenes or inactivation of antioncogenes[13]. Such oncogenic signals have been shown to further increase ROS generation. Moreover, the reduced expression of antioxidant enzymes in cancer cells brings about ROS accumulation. Exposure to the ionizing radiation is as well considered a factor contributing to the development of cancer by increasing the production of ROS. One of the approaches used to destroy the cancer cells is to enhance the ROS stress to a level beyond the threshold in the cell such that the cell undergoes apoptosis. This can be done by supplying the cell with anticancer agents that exhaust the antioxidant potential of the cell and at the same time increase ROS stress in the cell[31]. Another approach is to provide the cancer cells with antioxidant phytochemicals that nullify the effect of ROS.

These free radicals can act as metabolic carcinogens at various sites viz. oral cavity, larynx, lung, oesophagus, stomach, pancreas, colorectal site, cervix, bladder, prostate cells. Few antioxidants have been known to retard the growth of cancer cells. Vitamin E is reported to enhance antineoplastic activity because of its role in preventing lipid peroxidation; thereby it inhibits rapid proliferation of cell and hence slows the metastatic growth[32]. Vitamin E executes anticancer effects by blocking formation of nitrosamines and nullifies carcinogens by scavenging them or by enhancing detoxification process. Increased vitamin E intake has been shown to decrease ovarian[33] and skin carcinomas[34], and oral cancers[35]. Lower uptake of α-tocopherol is associated with greater risk of cervical cancer[36], prostate carcinoma[37], and lung cancer[38]. Dose of vitamin E, dosage cycles and the route of administration may be the factors that override the possible protective effects of vitamin E.

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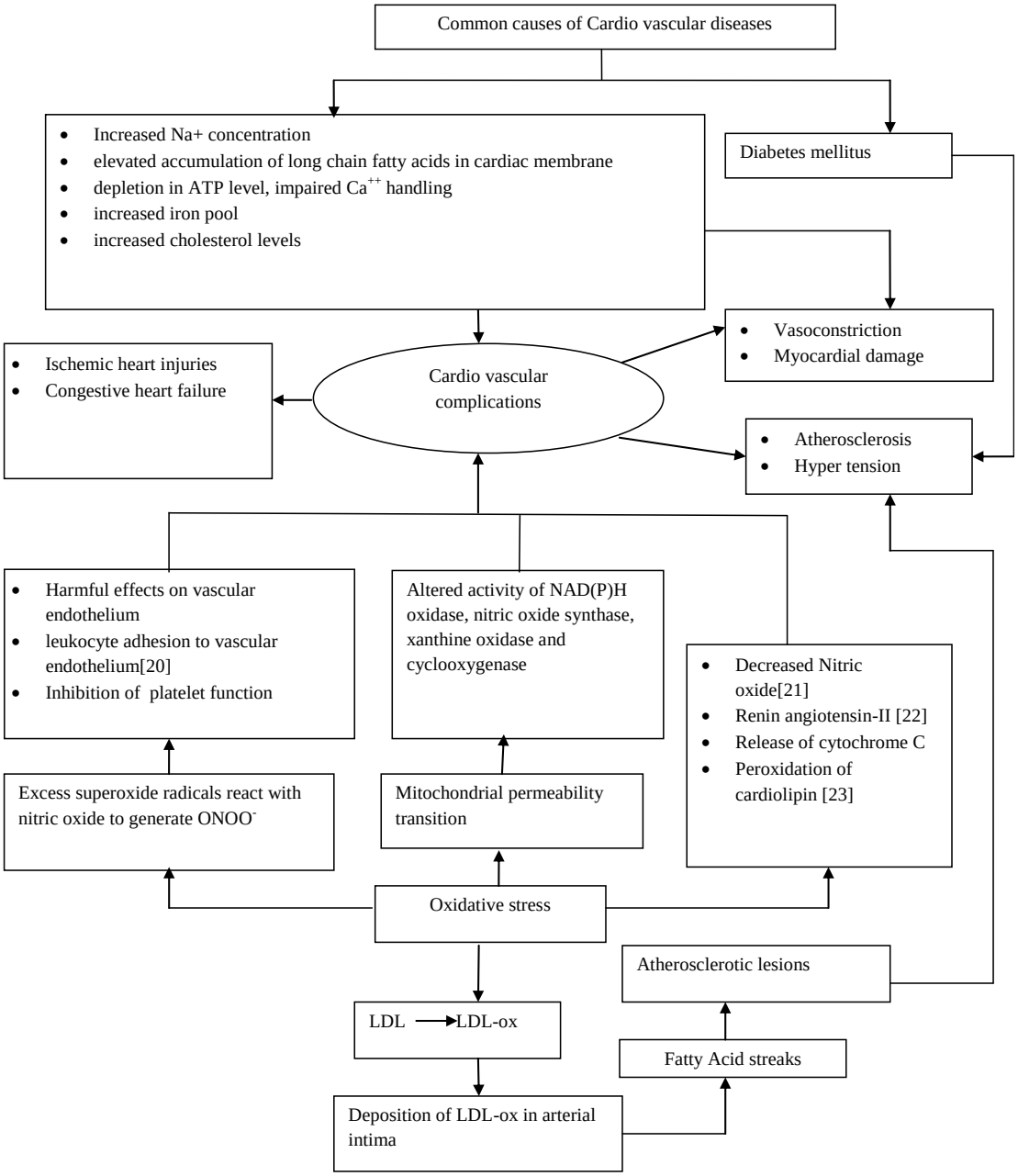

Figure 1: Common causes of cardio vascular diseases.

Ascorbic acid has also been observed to be potent against many types of cancers viz. oral cancer[39], larynx cancer[40], lung cancer[41], esophagus cancer, gastric cancer[42], colon cancer, rectal cancer[43] and cervical cancer[36]. On the other hand examples of pro-oxidant effects of ascorbic acid have also been quoted. Ascorbic acid is assumed to undergo oxidation under oxidative condition and produce pro-oxidant effects like promoting either novel mutations or mutations of tumor suppressor genes[44].

The best documented antioxidant action of carotenoids is their ability to quench singlet oxygen and prevent lipid peroxidation. β-carotene and lycopene have strong activity against prostate carcinoma. Several detriments of carotenoids have been reported. The increase in carotenoid concentration is related to the promotion of tumor growth with the most prevalent example of increased risk of lung carcinoma in patients with increased β-carotene supplementation[45]. 'ATBC' (The Alpha Tocopherol Beta Carotene Cancer Prevention Study) and 'CARET' (Combination of β-carotene and vitamin A on lung cancer and cardiovascular disease) are the widely cited trials indicating pro-oxidant effects of β-carotene[45]. β-carotene and other carotenoids exert antioxidant

activity depending on the system and carcinogen used to study them. The antioxidant activity of these compounds can shift to a pro-oxidant activity depending on factors such as oxygen tension and carotenoid actions. No single approach or study involving any particular population is adequate to provide a complete representation of complex relation between antioxidants and the risk of cancer. Several factors need to be accounted simultaneously before recommending any supplementation of antioxidants

NEURODEGENERATIVE DISEASES

Dysfunction of brain, spinal cord and peripheral nerves gives way to a number of degenerative diseases of the nervous system. High uptake of oxygen, relatively high levels of redox transition metals, increased levels of polyunsaturated fatty acid, lower levels of antioxidants and lower regeneration capacity are the factors that cumulatively cause oxidative damage in the brain. One of the major causes of oxidative stress in the brain is the production of free radicals as a byproduct during the metabolism of excitory amino acids and neurotransmitters, particulary glutamate[46] and some enzymes such as monoamine oxidase, tyrosine hydroxylase, L-amino acid oxidase and phospolipase A_2 on activation by NMDA receptor. When the level of iron in the tissue increases or when the axonal transport of iron is impaired, free iron gets deposited in the cells. This free iron in presence of ROS leads to neurodegeneration[47]. Release of pro-inflammatory and neurotoxic factors like TNF-α, IL-1, RNS and ROS by the activated microglia leads to neuronal damage[11]. A number of diseases have been proposed to be caused due to oxidative stress in the brain, e.g., Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS and "Lou Gehrig's disease"), demylenating disease, diabetic polyneuropathy, Down's syndrome, HIV neuropathy, Huntington's disease, multiple system atropy, Parkinson's disease, Prion's disease, stroke-ischemia reperfusion injury, tardive dyskensia (TD), traumatic brain injury, etc.

Parkinson's disease

PD is caused due to degeneration of dopamine neurons, which is initiated from oxidative damage of mitochondria, membrane and membrane structures, and proteins. Oxidative damage of cells in the brain of Parkinsonian patients is indicated by- increased lipid peroxidation of the membrane, decrease in concentration of antioxidants particulary glutathione, increase in iron concentration, and increase in the activity of manganese dependent SOD in the substantia nigra. ROS are observed to be produced in patients when given L-dihydroxyphenylalanine (L-dopa), one of the agent used in the treatment of PD. Multiple antioxidant therapy is also given to the patients in such cases[48].

Alzheimer's disease

AD is a common form of dementia. The patients with this disease show the increased production of free radicals due to inflammatory reactions. Cytokines, complementary proteins, free radicals and adhesion molecules generated at the inflammatory sites act as neurotoxins. The major hallmarks of Alzheimer's disease are the senile plaques composed largely of amyloid β peptides (Aβ) and neuronal aggregates of abnormally phosphorylated cytoskeletal proteins[7]. These Aβ proteins are the results of toxicity mediated by free radicals. Aβ proteins renders the nerve cells prone to further oxidative damage, interacts with various neurotransmitters and therefore affects neurotransmission and also disturbs intracellular Ca^{++} homeostasis[49]. Aβ protein is also reported to cause oxidative damage to the neuronal membrane and hence leads to cell lysis[50].

Amyotrophic lateral sclerosis

Mutations in gene encoding Cu-Zn-SOD, reduction in SOD activity, increase in protein carbonyl content (a measure of protein oxidation) and increase in 2-thiobarbituric reactive substances (products of lipid peroxidation) are the evidences proving the role of oxidative stress in neuron degeneration in patients suffering with ALS[51].

Others

Schizophernia patients too have shown increased levels of lipid peroxidation products and altered levels of both enzymatic and non-enzymatic oxidants[5] . Patients treated with neuroleptic drugs also show oxidative damage caused to the neurons which give rise to TD, a movement disorder[52]. Prion's disease or transmissible spongiform encephalopathies (TES) is a rare neurodegenerative disease which involves oxidative damage of neurons due to dramatic loss of antioxidant defences[53].

Antioxidant supplementation is found to be an effective therapy against many such neurodegenerative diseases. However, the passage of antioxidants through the blood - brain barrier poses a major challenge in the treatment of these diseases. Vitamin C and vitamin E have been reported to have protective effects when supplied in combination in patients with Parkinson's disease, whereas vitamin E alone was found to be ineffective. Vitamin A was not reported to have any effect. Several research groups have also reported vitamin E and vitamin C to have neuroprotective effects[54]. α-tocotrienol was found to exert the most potent neuroprotective effect among the different analogues of vitamin E as they can readily pass the blood-brain barrier[55]. Quercetin, a naturally occurring bioflavonoid was found to prevent oxidative damage to the neurons induced by neuroleptic drugs[56]. Apart from side effects of gastric upset by increased levels of vitamin C, no

detrimental effects of antioxidants on the neurons have been reported.

VIRAL INFECTIONS

At a limited concentration, ROS are involved in immune response against pathogens. However, higher level of ROS has been implicated in the pathogenesis of many viral infections. Invaded virus induces activation of phagocytosis which causes oxidative burst along with release of TNF-6 and IL-I having pro-oxidant effect from activated phagocytes and monocytes.

Viral infection influences the host cell pro/-anti oxidant balance by increasing level of nitric oxide along with inhibition of antioxidant enzyme such as SOD. Altered redox status of host may also cause mutation in viral genome and/or selection of mutant leading to increased viral replication. Deficiency of specific antioxidant may exert similar effect such as those observed with Coxsackie virus B3, whose non infectious strain was found to acquire ability to cause infection due to oxidative stress[57].

Influenza

Influenza virus titer increases due to effect of ROS on protease inhibitors of alveolar cells. Overall concentration of antioxidants such as α-tocopherol, vitamin C and glutathione is observed to decrease with increase in $NO^{\cdot}$, H_2O_2, lipid peroxides and lipofucsin treatment with SOD conjugated to either pyran or dextran, dimerized SOD, allopurinol and protease inhibitor have been shown to exert protective effects in influenza infected mice. Administration of quercetin is found to diminish the lipid peroxidation levels in influenza infected mice[58]. Also, administration of α-tocopherol, vitamin C and Se has been found to replenish level of redox homeostasis and decrease viral load.

Hepatitis

ROS generated hepatocarcinoma in hepatitis B viral infection is recognized to be similar to ROS induced chemical carcinoma. Also, increased lipid peroxidation has been observed in chronic hepatitis infection with increased TNF-α promoting viral replication. Indeed many studies have shown role of oxidative stress and lipid peroxidation in the fatty liver ultimately causing necroinflammation and necrosis of hepatic cells[59]. Alcohol induced hepatitis has a free radical associated pathogenesis. However, supplementations of α-tocopherol, Se and Zn have been found to decrease the levels of billirubin, ammonia and malondialdehyde (MDA), suggesting decrease in oxidative stress. Administration of catechin, NAC, TNF, combination of α-lipoic acid, silymarin and Se[60], B-complex, and ascorbic acid has led to favorable response in patients with chronic hepatitis infection[61].

HIV

Redox imbalance generated because of decrease in glutathione, Se, ubiquinone 10, ascorbic acid, increased lipid peroxidation and protein oxidation followed by HIV and SIV infection has been suggested as a causative factor for associated immunedeficiency and increased viral load. HIV-I infection indeed may lead to oxidative stress associated neuropathogenesis such as dementia[62], Alzeimer's disease[63], and ataxia telangiectasia[64] in patients. Such neuropathological conditions can occur due to oxidative stress in brain generated due to HIV infection

Use of antioxidants in curing HIV infection is exemplified by application of NAC that inhibits HIV induced apoptosis, and involvement of antioxidant pathway in Bcl-2 mediated inhibition of apoptosis[65]. Phase-I clinical trials[66] have suggested role of glutathione in maintaining redox balance[67]. Other antioxidants including ascorbic acid, α-tocopherol, dithiocarbamate, and α-lipoic acid has been found to be effective against HIV[65].

Along with major antioxidants cysteine derivatives, carotenoids[68], β-carotene[69], and vitamin-A[70] have been proposed as therapeutic agents in combating viral pathogenesis. Other viral infections enhanced by oxidative stress are - measles [71], pneumonitis[72], and those caused by Retrovirus ts-I[73], Epstein-Barr virus[65], adenovirus[65], Sendai virus[67], and coxsackie B virus [57]. In some of the studies, negative effects of antioxidant supplementation such as hypokalemia, mild exacerbation of hypertension and high systolic blood pressure have been observed[61]. Hence, further research is demanded before utilizing antioxidant therapy to combat viral infections.

AGEING

Ageing can be defined as sequential impairment of physiological functions after the reproductive phase of life of an organism, with declined ability to respond to a wide range of stress, increased risk of age associated diseases and increased likelihood of death[7]. Although, ageing is considered as a multifactorial process, oxidative damage to cellular components and mitochondrial dysfunction are the major factors contributing to the complex process of ageing.

Several evidences reveal that the increased levels of oxidized biomolecules including DNA, proteins, and lipids along with accumulation of oxidatively damaged cellular components, cataract formation and lipofuscin accumulation, accompany ageing. These damaged cellular products accumulate in the cell and hamper many cellular processes[74]. ROS mediated oxidation and/or peroxidation of lipid molecules causes

alteration in cellular and membrane function and accumulation of LDL-ox leading to age related diseases such as hypertension, atherosclerosis and other vascular and neurological disorders.

Mitochondria are devoid of histones and introns. ROS produced during oxidative phosphorylation is in the vicinity of mitochondrial DNA and so the latter is highly susceptible to oxidative damage. Besides, the mitochondria lack DNA repair enzymes. All these factors together damage the mitochondrial DNA. Somatic mutations in the mitochondrial DNA are a major contributor to human ageing and associated diseases[75]. Insufficient activity of proteolytic enzymes that hydrolyze oxidized proteins formed due to oxidative damage, oxidative burst from phagocytes and oxidation of redox-responsive signaling protein leading to dysregulation of signaling process are the other factors responsible for cell degeneration and ageing. To compensate the damage caused by increased mitochondrial DNA lesions, cell increases mitochondrial turnover leading to the production of more oxidants. These factors cumulatively decrease the bioenergetic function in human liver, and promote, age related decrease in mitochondrial respiration and mitochondrial transcription rate during ageing and associated complications.

Capacity of antioxidant system of tissues declines with age, leading to gradual loss of oxidant-antioxidant balance. Supply of antioxidants exogenously to combat ROS may delay or decrease the complications associated with ageing and improve health. For example, oral administration of antioxidants protected mouse and rat from glutathione oxidation and mitochondrial damage[75]. Also supplementation of elderly with α-tocopherol has been found to increase resistance to oxidative damage and delay onset of ageing[76], cancer[28- 3333-38]and coronary artery diseases[2226]. A small molecule N_6-furfuryladenine or kinetin has been shown to have considerable anti-ageing effects on human cells due to its direct action as an antioxidant[76]. However, its use is limited to cosmetics[77]. Supplements of adequate amount of antioxidants from early age can be helpful to preserve health in prematurely ageing population.

Evidences suggest role of dietary vitamin E in protection against oxidative damage to DNA in human lymphocytes and WBC with suppression of LDL-oxidation *in vivo* and *in vitro*[78]. Although the role of antioxidants in increasing lifespan is not yet been established, beneficial effects of antioxidants on ageing and associated disorders still remains a sensible possibility[75]. In any case, diet rich in natural antioxidants (fruits and vegetables) remains recommendable.

MALE INFERTILITY

Oxidative stress in the reproductive tract is reported to exert harmful effects on sperm number, motility, quality and function including damage to sperm nuclear DNA [779]. Low physiological levels of ROS, however, are found to be essential for the regulation of normal sperm functions such as sperm capacitation, acrosome reaction and sperm-oocyte fusion[80] and to acquire fertilizing capacities. The reactive species in the mammalian spermatozoa have been shown to peroxidize PUFA chain in the membrane, inhibit glucose-6-phosphatase[81], bring about DNA fragmentation[82] and also disrupt the inner and outer mitochondrial membrane resulting in release of cytochrome-C and associated caspases of apoptosis[81], leucocytopenia, genital tract infection, vericocele, teratozoospermia, astheriozoospermia, azoospermia and fenton chemistry associated Fe^{+2} generation[83].

Available evidences suggest the use of antioxidants in managing selective cases of male infertility. Few clinical trials have reported the beneficial effects of antioxidant administration on sperm DNA integrity [84]. Vitamin C (200 mg/day), itamin E (200 mg/day) and GSH (400 mg/day) have been shown to decrease 8-hydroxy-deoxyguanine levels[84]. However, other clinical trials fail to demonstrate similar benefits. Both *in vitro* and *in vivo* studies[85] have proven that vitamin C and vitamin E could protect the DNA from oxidative damage when given in combination, but vitamin C has been shown to act as pro-oxidant when given separately. Hence, supplementation with vitamin E[86], vitamin B[87], vitamin C[88], GSH[89], and cornitine[90] have been shown to reduce MDA concentration and influence sperm motility and fertility in dose dependent manner.

CYSTIC FIBROSIS

Cystic fibrosis is a genetic disorder that involves dysfunction of the CFTM (cystic fibrosis transmembrane) protein and result in progressive respiratory failure with chronic pulmonary oxidative failure. Increased oxidative stress in cystic fibrosis is suggested by elevated plasma MDA levels , elevated breath pentane and ethane, elevated plasma levels of H_2O_2, depletion of antioxidant defences including low level of GSH, vitamin E, vitamin C in plasma, depletion of the major lipoperoxidation substrates such as linoleic acid and archidonic acid. In case of bacterial infection in the lungs, there is infiltration of neutrophils which try to destroy the invading pathogen by releasing high concentration of ROS. The ROS generated as a result of this immune response, leak into the surrounding cells causing lung tissue damage. Other sources of oxidative stress involve increased metabolic rate, increased oxygen uptake[91], reduced antioxidative protection and mitochondrial

dysfunction. In patients with cystic fibrosis, balance between pro-oxidant/antioxidant process is disturbed[92] due to lack of dietary antioxidants such as vitamin E and carotenoids.

METABOLIC ENGINEERING OF ANTIOXIDANT SYNTHESIS IN PLANTS

In view of the advantages of natural antioxidants over synthetic ones, it seems reasonable to enhance the synthesis of antioxidants in plants by the way of metabolic engineering. Few efficient approaches are available to increase production of antioxidants in plants. Tocopherol group of antioxidants are particularly low in the plant parts and so various experiments have been conducted for the engineering of tocochamanols (tocopherols and tocotrienols). In one such experiment, the tocotrienol content of soybean seeds was increased by 95% which initially contained only traces of it[93]. The α-tocopherol content in *Arabidopsis* seeds was increased by overexpression of γ-tocopherol methyl transferase, the final enzyme of the pathway for α-tocopherol synthesis[94]. Expression of HGGT (homogentisate geranyl geranyl diphosphate) c-DNAs from barley, wheat and rice in tobacco callus and *Arabidopsis* leaves resulted in an increased production of tocotrienol by 10-15 folds[95]. Also, a novel approach involving microorganisms (*Escherichia coli*) have been reported for anthocyanin biosynthesis[96]. Increased carotenoid accumulation in association with the formation of metabolic sink structures in the transgenic crops offers another approach to increase carotenoid content. Seventy eight-fold increase in total fruit flavonoids was achieved through ectopic expression of single biosynthetic enzyme chalcone isomerase in tomatoes[97]Metabolic engineering for increasing concentration of natural antioxidants in plants will certainly improve the nutritional as well as therapeutic value of plant products.

CONCLUDING REMARKS

Consumption of dietary antioxidants that are present in fruits and vegetables is associated with a lowered risk of degenerative diseases. Epidemiological studies have shown strong association of the effect of dietary antioxidants with the effect of other important vitamins and ingredients in fruits and vegetables. Nevertheless, the antioxidant content of fruits and vegetables is a major contributor to their protective effect. It may be that such simple and cost-effective measures as improving diet or supplementation with a number of key antioxidants can dramatically improve the health of the general population and the individual person. But at the same time, the dose and route of administration of antioxidants are important factors to be considered before taking in any exogenous supplements of antioxidants. Many research groups are focusing on the appraisal of antioxidant properties of various parts of plants. Metabolic engineering of plants can improve the yield of antioxidants. In order to identify the compatible targets for metabolic engineering, screening of all known plant species for their antioxidant activity needs to be done. More research on natural antioxidants can certainly help in increasing our average life expectancy in the coming decades.

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