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Review Article

# ANTIOXIDANTS AS IMMUNOMODULATOR: AN EXPANDING RESEARCH AVENUE

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**ABSTRACT**

Free radicals and related species have attracted a great deal of attention in recent years. They are mainly derived from reactive oxygen and nitrogen species. They are generated in our body by various endogenous systems, exposure to different physicochemical conditions or pathophysiological states. Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations. Free radical damage to protein can result in loss of enzyme activity. Damage caused to DNA, can result in mutagenesis and carcinogenesis. This article presents current understanding of the role of free radicals and oxidative stress in pathogenesis of various diseases and advancements made in developing antioxidant based therapeutics and also discuss the opportunities to develop therapeutics from traditional medicinal practice.

**Key words:** Antioxidant, Immunomodulator, Free radicals, Therapeutic approaches.

## INTRODUCTION

As antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radicals intermediates, and inhibit other oxidation agents such as thiols,ascorbic acid or polyphenols (Sies *et al*.,1997). Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase,superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition, or inhibition of the antioxidants enzymes, cause oxidative stress and may damage or kill cells.As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease (Bjelakovic *et al*., 2007).

### Free radicals and their role in diseases

Free radicals are natural by-products of our own metabolism. These are electrically charged molecules that attack our cells, tearing through cellular membranes to react and create havoc with the nucleic acids, proteins, and enzymes present in the body. These attacks by free radicals,

collectively known as oxidative stress, are capable of causing cells to lose their structure, function and can eventually destroy them. They are continuously produced by our body's use of oxygen such as in respiration and some cell-mediated immune functions. They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air-pollution, pesticides, etc. (Li and Trush, 1994). Normally there is a balance between the amount of free radicals generated in the body and the antioxidant defense systems that scavenge/quench these free radicals preventing them from causing deleterious effects in the body (Nose, 2000). The antioxidant defense systems in the body can only protect the body when the amount of the free radicals is within the normal physiological level. But when this balance is shifted towards more of free radicals, increasing their burden in the body either due to environmental condition or produced within the body, it leads to oxidative stress, which may result in tissue injury and subsequent diseases (Finkel and Holbrook, 2000). Since free radicals play such an important role in the disease scenario of an individual, a thorough understanding of the various physiologically significant free radicals is of paramount importance before the search of the radical scavengers or the antioxidant principles to treat the physiological disorders caused by them. Free radicals may be designated as molecular sharks that damage molecules in cell membranes, mitochondria (the cell's energy plants), DNA (the cell's intelligence) and are very unstable, tend to rob electrons from the molecules in the immediate surrounding in order to replace their own losses. Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals (O2 •−, and •OH) but also some non-radical derivatives of oxygen. These include hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl) and ozone ($O_3$) (Bandhopadhyay *et al*., 1999). Over about 100 disorders like rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal ulcerogenesis and AIDS have been reported as ROS mediated. Some specific examples of ROS mediated diseases include Alzheimers disease, Parkinson's disease, Atherosclerosis, Cancer, Down's syndrome and ischemic reperfusion injury in different tissues including heart, liver, brain, kidney and gastro intestinal tract. The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of ageing. The role of radicals in various diseases is dealt in detail.

### Antioxidant defense

It is evident through the reactions of oxygen, that it is toxic; still only the aerobes survive its presence, primarily because they have evolved an inbuilt antioxidant defense. Antioxidant defenses comprise:

- Agents that catalytically remove free radicals and other reactive species like SOD, CAT, peroxidase and thio specific antioxidants.
- Proteins that minimize the availability of peroxidase such as iron ions, copper ions and haem.
- Proteins that protect biomolecules against oxidative damage example heat shock proteins.
- Low molecular mass agents that scavenge ROS and RNS, example GSH, ascorbic acid, tocopherol.

The antioxidants may be defined as "any substance, when present at low concentrations compared with that of an oxidizable substrate that significantly delays or prevents oxidations of that substrate". The term oxidizable substrate includes every type of molecule found *in vivo*. Antioxidant defense include the antioxidant enzymes like SOD, CAT, GSH-px, etc, low molecular agents and dietary antioxidants (Halliwell and Gutteridge., 1999).

**Significance of antioxidants in relation to disease**

Antioxidants may prevent and/or improve different diseased states (Knight, 2000). Zinc is an essential trace element, being a co-factor for about 200 human enzymes, including the cytoplasmic antioxidant Cu-Zn SOD, isoenzyme of SOD mainly present in cytosol. Selenium is also an essential trace element and a co-factor for glutathione peroxidase. Vitamin E and tocotrienols (such as those from palm oil) are efficient lipid soluble antioxidants that function as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including LDL (Packer *et al*., 1998; Kagan *et al*. 2002).

Vitamin E is considered as the 'standard antioxidant' to which other compounds with antioxidant activities are compared, especially in terms of its biological activity and clinical relevance. The daily dietary allowance varies between 400 IU to 800 IU. Vitamin C (ascorbic acid) is a water-soluble free radical scavenger. The daily recommended dietary allowance is 60 mg. A part from these carotenoids such as beta-carotene, lycopene, lutein and other carotenoids function as important antioxidants and they quench $O_2$ and ROO•. Flavonoids, mainly present as colouring pigments in plants also function as potent antioxidants at various levels (Sies., 1996; Cadenas and Packer., 1996; Kagan *et al*., 2002).

**Correlation between enhancement of ATP generation capacity and antioxidative capacity**

Mitochondrial oxidative phosphorylation generates ROS as byproducts. Highly reactive chemically, ROS attack cellular structures located near the sites where ROS are generated. Mitochondrial DNA, proteins, and lipids in the inner membrane of mitochondria are thus vulnerable to oxidative damage (Leeuwenburgh *et al.,* 1999), resulting in generalized organelle dysfunction, defective mitochondrial biosynthesis and poor energy metabolism (Chandwaney *et al*., 1998). Under normal physiological conditions, the mitochondrial antioxidant defense system adequately handles the potentially detrimental effects of ROS derived from energy metabolism (Halliwell *et al*., 1996). When a functional imbalance between ROS levels and antioxidant concentrations caused by various disease states and/or aging occurs, age-related disorders such as cancer, cardiovascular diseases, brain dysfunction, or cataract may occur (Finkel *et al*., 2000). Antioxidant supplementation, particularly from herbal extracts, has become a trend in preventive health care.

**Antioxidants and protection against human disease**

There are a number of epidemiological studies that have shown inverse correlation between the levels of established antioxidants / phytonutrients present in tissue / blood samples and occurrence of cardiovascular disease, cancer or mortality due to these diseases. However, some recent metaanalysis show that supplementation with mainly single antioxidants may not be that effective (Vivekananthan *et al*. 2003), a view that contrasts with those of preclinical and epidemiological studies on consumption of antioxidant-rich foods. Based on the majority of epidemiological and case control studies recommendations were made for the daily dietary intake of some established antioxidants like vitamin E and C as well as others.

Requirement for antioxidants in Indian conditions differ from that of industrialized western countries due to the nutritional differences. There are also a number of dietary supplements rich in antioxidants tested for their efficacy. There are many laboratories from India working on the antioxidant effect of plant compounds, mainly derived from natural sources that are capable of protecting against such damage. Such studies show that compounds with potent antioxidant activity include carotenoids, curcumin from turmeric, flavonoids, caffeine present in coffee, tea, etc., orientin, vicenin, glabridin, glycyrrhizin, emblicanin, punigluconin, pedunculagin, 2-hydroxy-4-methoxy benzoic acid, dehydrozingerone, picroliv, withaferin, yakuchinone, gingerol, chlorogenic acid, vanillin (food flavouring agent) and chlorophyllin (Devasagayam *et al*., 2004).

**Uses in technology**

**Industrial uses**

Antioxidants are frequently added t industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidation, and in gasolines to prevent the polymerization that leads to the formation of engine-fouling residues (Boozer *et al*, 1955).

**Table 1: ref wwww.innospecinc.com/americas/products/fuel/antioxidants. cfmo**

| Fuel additive | Components | Applications |
|---|---|---|
| AO-22 | N,N'-di-2-butyl-1,4-phenylenediamine | Turbine oils, transformer oils, hydraulicfluids, waxes, and greases |
| AO-24 | N,N'-di-2-butyl-1,4-phenylenediamine | Low-temperature oils |
| AO-29 | 2,6-di-tert-butyl-4-methylphenol | Turbine oils, transformer oils, hydraulic fluids, waxes, greases, and gasolines |
| AO-30 | 2,4-dimethyl-6-tert-butylphenol | Jet fuels and gasolines, including aviation gasolines |
| AO-31 | 2,4-dimethyl-6-tert-butylphenol | Jet fuels and gasolines, including aviation gasolines |
| AO-32 | 2,4-dimethyl-6-tert-butylphenol and 2,6-di-tert-butyl-4-methylphenol | Jet fuels and gasolines, including aviation gasolines |
| AO-37 | 2,6-di-tert-butylphenol | Jet fuels and gasolines, widely approved for aviation fuels |

**Food preservatives**

Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors (Kader *et al*., 1989). Consequently, packaging of fresh fruits and vegetables contains an ~8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food (Zallen *et al*., 1975). These preservatives include natural antioxidants such as ascorbic acid (AA, E300) and tocopherols (E306), as well as synthetic antioxidants such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321) (Iverson *et al.,*1995).

**Newer therapeutic approaches using antioxidants**

Antioxidant-based drugs/formulations for prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease (AD), Parkinson's disease, cancer, etc. appeared over the past three decades. Free radical theory has greatly stimulated interest in the role of dietary antioxidants in preventing many human diseases, including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration and diabetes.

Dietary antioxidants may have promising therapeutic potential in delaying the onset as well as in preventing the ageing population and its related complications. Two neuroprotective clinical trials are available with antioxidants: Deprenyl and tocopherol antioxidant therapy of Parkinson's study. It has embarked on a fast track

programme to discover new drugs by building on traditional medicines and screening the diverse plants and microbial sources of the country. In terms of its size, diversity and access to talent and resources this programme is not only the world's largest project of its kind, but is also unique (Jayaraman., 2003).

Free radicals have been implicated in the etiology of large number of major diseases. They can adversely alter many crucial biological molecules leading to loss of form and function. Such undesirable changes in the body can lead to diseased conditions. Antioxidants can protect against the damage induced by free radicals acting at various levels. Dietary and other components of plants form major sources of antioxidants. The relation between free radicals, antioxidants and functioning of various organs and organ systems is highly complex and the discovery of 'redox signaling' is a milestone in this crucial relationship. Recent research centers on various strategies to protect crucial tissues and organs against oxidative damage induced by free radicals. Many novel approaches are made and significant findings have come to light in the last few years. The traditional Indian diet, spices and medicinal plants are rich sources of natural antioxidants. Higher intake of foods with functional attributes including high level of antioxidants in functional foods is one strategy that is gaining importance in advanced countries and is making its appearance in our country. Coordinated research involving biomedical scientists, nutritionists and physicians can make significant difference to human health in the coming decades. Research on free radicals and antioxidants involving these is one such effort in the right direction ((Devasagayam *et al*., 2004).

**CONCLUSION**

Free radicals have been implicated in the etiology of large number of major diseases. They can adversely alter many crucial biological molecules leading to loss of form and function. Such undesirable changes in the body can lead to diseased conditions. Antioxidants can protect against the damage induced by free radicals acting at various levels. Dietary and other components of plants form major sources of antioxidants. The relation between free radicals, antioxidants and functioning of various organs and organ systems is highly complex and the discovery of redox signaling is a milestone in this crucial relationship. Recent research centers on various strategies to protect crucial tissues and organs against oxidative damage induced by free radicals. Many novel approaches are made and significant findings have come to light in the last few years. The traditional Indian diet, spices and medicinal plants are rich sources of natural antioxidants. Higher intake of foods with functional attributes including high level of antioxidants in functional foods is one strategy that is gaining importance in advanced countries and is making its appearance in cosmos.

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