

# Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects

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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 oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS), and 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. Redox signaling is a major area of free radical research that is attracting attention. Nature has endowed us with protective antioxidant mechanisms- superoxide dismutase (SOD), catalase, glutathione, glutathione peroxidases and reductase, vitamin E (tocopherols and tocotrienols), vitamin C etc., apart from many dietary components. There are epidemiological evidences correlating higher intake of components/ foods with antioxidant abilities to lower incidence of various human morbidities or mortalities. Current research reveals the different potential applications of antioxidant/free radical manipulations in prevention or control of disease. Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity. Newer and future approaches include gene therapy to produce more antioxidants in the body, genetically engineered plant products with higher level of antioxidants, synthetic antioxidant enzymes (SOD mimics), novel biomolecules and the use of functional foods enriched with antioxidants. ©

## INTRODUCTION AND BASICS OF FREE RADICAL RESEARCH

In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants. So it will be pertinent to examine the possible role of 'free radicals' in disease and 'antioxidants' in its prevention, especially the current status of the subject matter and future prospects, in this review.

### Free Radicals- Friends or Foes?

The events of World War II (1939-1945) led directly to the birth of free radical biochemistry. The two atom bombs (6th August 1945, Hiroshima and 9th August 1945, Nagasaki) led

to massive deaths to entire population, and the survivors had shortened life-span. In 1954, Gershman and Gilbert speculated that the lethal effects of ionizing radiation might be ascribed to formation of reactive oxygen species (ROS). Since then free radicals (atoms with an unpaired electron) such as ROS and reactive nitrogen species (RNS) have gained notoriety. (Gilbert *et al*, 1981).<sup>1</sup> In popular scientific/biomedical literature the term 'free radical' is used in a broad sense and also includes related reactive species such as 'excited states' that lead to free radical generation or those species that results from free radical reactions. In general, free radicals are very short lived, with half-lives in milli-, micro- or nanoseconds. Details about some of the biologically important reactive species are presented as Table 1. Free radicals have been implicated in the etiology of several human diseases as well as ageing (Harman, 1958; Halliwell and Gutteridge, 1997).<sup>2,3</sup> But it has to be emphasized that ROS and RNS are both produced in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. Most cells can produce superoxide (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and

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**Table 1: Reactive oxygen and nitrogen species of biological interest**

| Reactive species                  | Symbol                        | Half life (in sec) | Reactivity / Remarks  |
|-----------------------------------|-------------------------------|--------------------|---|
| <b>Reactive oxygen species :</b>  |                               |                    |   |
| Superoxide                        | O <sub>2</sub> <sup>•-</sup>  | 10 <sup>-6</sup> s | Generated in mitochondria, in cardiovascular system and others  |
| Hydroxyl radical                  | •OH                           | 10 <sup>-9</sup> s | Very highly reactive, generated during iron overload and such conditions in our body                        |
| Hydrogen peroxide                 | H <sub>2</sub> O <sub>2</sub> | stable             | Formed in our body by large number of reactions and yields potent species like •OH                          |
| Peroxyl radical                   | ROO•                          | s                  | Reactive and formed from lipids, proteins, DNA, sugars etc. during oxidative damage                         |
| Organic hydroperoxide             | ROOH                          | stable             | Reacts with transient metal ions to yield reactive species  |
| Singlet oxygen                    | <sup>1</sup> O <sub>2</sub>   | 10 <sup>-6</sup> s | Highly reactive, formed during photosensitization and chemical reactions                                    |
| Ozone                             | O <sub>3</sub>                | s                  | Present as an atmospheric pollutant, can react with various molecules, yielding <sup>1</sup> O <sub>2</sub> |
| <b>Reactive nitrogen species:</b> |                               |                    |   |
| Nitric oxide                      | NO•                           | s                  | Neurotransmitter and blood pressure regulator, can yield potent oxidants during pathological states         |
| Peroxynitrite                     | ONOO•                         | 10 <sup>-3</sup> s | Formed from NO. and superoxide, highly reactive   |
| Peroxynitrous acid                | ONOOH                         | fairly stable      | Protonated form of ONOO•  |
| Nitrogen dioxide                  | NO <sub>2</sub>               | s                  | Formed during atmospheric pollution   |

nitric oxide (NO) on demand. Hence, it is worth emphasizing the important beneficial role of free radicals.

1. Generation of ATP (universal energy currency) from ADP in the mitochondria: oxidative phosphorylation
2. Detoxification of xenobiotics by Cytochrome P450 (oxidizing enzymes)
3. Apoptosis of effete or defective cells
4. Killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes
5. Oxygenases (eg. COX: cyclo-oxygenases, LOX: lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

In recent years, it has become increasingly clear the ROS, such as O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub> may act as second messengers. Observations made some twenty years ago had suggested that ROS may play a role in modulating cellular function. Studies done then revealed that exogenous H<sub>2</sub>O<sub>2</sub> could mimic the action of the insulin growth factor. The discovery of redox-sensitive transcription factors and that NO<sup>•</sup>, a free radical produced enzymatically, plays a physiological role in vasodilation and neurotransmission through activation of soluble guanylated cyclase further supported the concept that ROS and RNS can act as second messengers to modulate signaling pathways. This led to the renaissance of the field

of redox signaling and with the accumulation of data in various systems, a clearer picture is emerging of the signaling pathways and specific targets affected by ROS/RNS (Yoshikawa *et al.*, 2000).<sup>4</sup>

Other sources of free radicals include redox cycling of xenobiotics, exposure to physicochemical agents like ionizing radiations such as X-rays and  $\gamma$ -rays besides visible light or UV in the presence of oxygen and an endogenous compound or a drug that act as photosensitizer. Most of the damage induced by ionizing radiations in biological systems is indirect and is mediated by products of radiolysis of water including hydrogen radical (•H), •OH, hydrated electron (e<sub>aq</sub><sup>-</sup>), H<sub>2</sub>O<sub>2</sub>, peroxy radical (ROO•), O<sub>2</sub><sup>•-</sup>, <sup>1</sup>O<sub>2</sub> etc. (Von Sonntag, 1987; Devasagayam and Kesavan, 1996).<sup>5,6</sup> Cigarette smoke contains a large amount of reactive species (Devasagayam and Kamat, 2002).<sup>7</sup> Cigarette tar contains quinone-hydroquinone-semiquinone system which reduces O<sub>2</sub> to form O<sub>2</sub><sup>•-</sup>, H<sub>2</sub>O<sub>2</sub> and •OH, while cigarette smoke contains small oxygen- and carbon-centered radicals as well as active oxidants such as NO<sup>•</sup> and nitrogen dioxide (NO<sub>2</sub>). Recent studies by Wentworth *et al.* (2003)<sup>8</sup> showed that antibodies, regardless of origin or antigenic specificity, could convert <sup>1</sup>O<sub>2</sub> into H<sub>2</sub>O<sub>2</sub> via a process that they have postulated to involve dihydrogen trioxide (H<sub>2</sub>O<sub>3</sub>). During ischemia-reperfusion, oxidants like O<sub>2</sub><sup>•-</sup>, •OH and H<sub>2</sub>O<sub>2</sub> are produced. This occurs during non-fatal myocardial infarction, surgeries, stroke, transplantation, blockage of arteries under pathological conditions, etc. During ischemia in the heart (in myocyte mitochondria) conversion of ATP to adenosine causes the generation of O<sub>2</sub><sup>•-</sup>, while in the blood vessels (endothelium) the pathway involved is the transition from xanthine to uric acid (Yoshikawa *et al.* 2000).<sup>4</sup>

### Antioxidants

'Antioxidants' are substances that neutralize free radicals or their actions (Sies, 1996).<sup>9</sup> Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell.  $\alpha$ -Tocopherol (vitamin E) is an essential nutrient which functions as a chain-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also part of the normal protecting mechanism. Other non-enzymatic antioxidants include carotenoids, flavonoids and related polyphenols,  $\alpha$ -lipoic acid, glutathione etc.

### Levels of Antioxidant Action

Antioxidants, capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention, interception and repair (see Figure 1 for details). Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase (SOD) that catalyses the dismutation of superoxide to H<sub>2</sub>O<sub>2</sub> and catalase that breaks it down to water (Sies, 1996; Cadenas and Packer, 1996).<sup>9,10</sup> Interception of free radicals is mainly by radical scavenging, while at the secondary level scavenging of

peroxyl radicals are effected. The effectors include various antioxidants like vitamins C and E, glutathione, other thiol compounds, carotenoids, flavonoids, etc. At the repair and reconstitution level, mainly repair enzymes are involved (Sies, 1996; Cadenas and Packer, 1996; Halliwell and Aruoma, 1993).<sup>9-11</sup>

### Concept of Oxidative Stress

The relation between free radicals and disease can be explained by the concept of 'oxidative stress' elaborated by Sies (1986).<sup>12</sup> In a normal healthy human body, the generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various levels of antioxidant defense. However, when it gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, cigarette smoking, ultraviolet rays, radiation, toxic chemicals, overnutrition and advanced glycation end products (AGEs) in diabetes, this delicately maintained balance is shifted in favor of pro-oxidants resulting in 'oxidative stress'. It has been implicated in the etiology of several (>100) of human diseases and in the process of ageing.

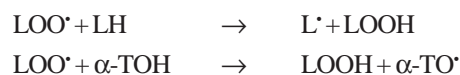
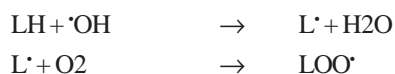
### Molecular damage induced by free radicals

All the biological molecules present in our body are at risk of being attacked by free radicals. Such damaged molecules can impair cell functions and even lead to cell death eventually resulting in diseased states.

### Lipids and Lipid Peroxidation

Membrane lipids present in subcellular organelles are highly susceptible to free radical damage. Lipids when reacted with free radicals can undergo the highly damaging chain reaction of lipid peroxidation (LP) leading to both direct and indirect effects. During LP a large number of toxic byproducts are also formed that can have effects at a site away from the area of generation, behaving as 'second messengers'. The damage caused by LP is highly detrimental to the functioning of the cell (Devasagayam *et al* 2003).<sup>13</sup>

Lipid peroxidation is a free radical mediated process. Initiation of a peroxidative sequence is due to the attack by any species, which can abstract a hydrogen atom from a methylene group (CH<sub>2</sub>), leaving behind an unpaired electron on the carbon atom (·CH). The resultant carbon radical is stabilized by molecular rearrangement to produce a conjugated diene, which then can react with an oxygen molecule to give a lipid peroxyl radical (LOO·). These radicals can further abstract hydrogen atoms from other lipid molecules to form lipid hydroperoxides (LOOH) and at the same time propagate LP further. The peroxidation reaction can be terminated by a number of reactions. The major one involves the reaction of LOO· or lipid radical (L·) with a molecule of antioxidant such as vitamin E or α-tocopherol (α-TOH) forming more stable tocopherol phenoxyl radical that is not involved in further chain reactions. This can be 'recycled' by other cellular antioxidants such as vitamin C or GSH.



The process of LP, gives rise to many products of toxicological interest like malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and various 2-alkenals. Isoprostanes are unique products of lipid peroxidation of arachidonic acid and recently tests such as mass spectrometry and ELISA-assay kits are available to detect isoprostanes (Yoshikawa *et al.* 2000).<sup>4</sup>

### Carbohydrates

Free radicals such as ·OH react with carbohydrates by randomly abstracting a hydrogen atom from one of the carbon atoms, producing a carbon-centered radical. This leads to chain breaks in important molecules like hyaluronic acid. In the synovial fluid surrounding joints, an accumulation and activation of neutrophils during inflammation produces significant amounts of oxyradicals, that is also being implicated in rheumatoid arthritis.

### DNA

Oxidative damage to DNA is a result of interaction of DNA with ROS or RNS. Free radicals such as ·OH, eaq- and H· react with DNA by addition to bases or abstractions of hydrogen atoms from the sugar moiety. The C4-C5 double bond of pyrimidine is particularly sensitive to attack by ·OH, generating a spectrum of oxidative pyrimidine damage products, including thymine glycol, uracil glycol, urea residue, 5-hydroxydeoxyuridine, 5-hydroxydeoxycytidine, hydantoin and others. Similarly, interaction of ·OH with purines will generate 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxydeoxyadenosine, formamidopyrimidines and other less characterized purine oxidative products. Several repair pathways repair DNA damage (Halliwell and Aruoma, 1993).<sup>11</sup> 8-OHdG has been implicated in carcinogenesis and is considered a reliable marker for oxidative DNA damage.

### Proteins

Oxidation of proteins by ROS/RNS can generate a range of stable as well as reactive products such as protein hydroperoxides that can generate additional radicals particularly upon interaction with transition metal ions. Although most oxidised proteins that are functionally inactive are rapidly removed, some can gradually accumulate with time and thereby contribute to the damage associated with ageing as well as various diseases. Lipofuscin, an aggregate of peroxidized lipids and proteins accumulates in lysosomes of aged cells and brain cells of patients with Alzheimer's disease (Stadtman, 1992).<sup>14</sup>

### Significance of antioxidants in relation to disease

Antioxidants may prevent and/or improve different diseased states (Knight, 2000).<sup>15</sup> 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 and Ong, 1998; Kagan *et al.* 2002).<sup>16,17</sup>

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. Apart from these carotenoids such as beta-carotene, lycopene, lutein and other carotenoids function as important antioxidants and they quench  $^1O_2$  and  $ROO\cdot$ . 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).<sup>9,10,17</sup>

#### **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 (Vivekanathan *et al.* 2003),<sup>18</sup> 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 (a water-soluble analogue of chlorophyll). (For more details see Table 2).

#### **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 with AD and its related complications. Two neuroprotective clinical trials are available with antioxidants: Deprenyl and tocopherol antioxidant therapy of Parkinson's study.

By fusing ancient wisdom and modern science, India can create world-class products. Therefore, 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).<sup>19</sup>

#### **Ayurveda, antioxidants and therapeutics**

Employing a unique holistic approach, Ayurvedic medicines are usually customized to an individual constitution. Ayurvedic Indian and traditional Chinese systems are living 'great traditions' and have important roles in bioprospecting of new medicines from medicinal plants, which are also rich sources of antioxidants. Current estimate indicates that about 80% of people in developing countries still rely on traditional medicine-based largely on various species of plants and animals for their primary healthcare. Ayurveda remains one of the most ancient and yet living traditions practiced widely in India.

#### **Sources of antioxidants, phytonutrients and functional foods**

Natural compounds, especially derived from dietary sources provide a large number of antioxidants (Table 3). Some beverages such as tea are also rich sources of antioxidants. A growing body of evidence suggests that moderate consumption of tea may protect against several forms of cancer, cardiovascular diseases, the formation of kidney stones, bacterial infections, and dental cavities. Tea is particularly rich in catechins, of which epigallocatechin gallate (EGCG) is the most abundant.

#### **Indian Medicinal Plants**

Apart from the dietary sources, Indian medicinal plants also provide antioxidants and these include: (with common/ayurvedic names in brackets) *Aegle marmelos* (Bengal quince, Bel), *Allium cepa* (Onion), *Allium sativum* (Garlic, Lahsuna), *Aloe vera* (Indian aloe, Ghritkumari), *Amomum subulatum* (Greater cardamom, Bari elachi), *Andrographis paniculata* (The creat, Kiryat), *Asparagus racemosus* (Shatavari), *Azadirachta indica* (Neem, Nimba), *Bacopa monniera* (Brahmi), *Camellia sinensis* (Green tea), *Cinnamomum verum* (Cinnamon), *Cinnamomum tamala* (Tejpat), *Curcuma longa* (Turmeric, Haridra), *Embllica officinalis* (Indian gooseberry, Amlaki), *Glycyrrhiza glabra* (Yashtimadhu), *Hemidesmus indicus* (Indian Sarasparilla, Anantamul), *Momordica charantia* (Bitter gourd), *Nigella sativa* (Black cumin), *Ocimum sanctum* (Holy basil, Tulsi), *Picrorrhiza kurroa* (Katuka), *Plumbago zeylanica* (Chitrak), *Syzygium cumini* (Jamun), *Terminalia bellarica* (Behda), *Tinospora cordifolia*

**Table 2 : Epidemiological studies on antioxidants in humans**

| Disease                                    | Antioxidant  | Parameter   | Effect                                |
|--|--|---|---------------------------------------|
| <i>Cancer</i>                              |  |   |                                       |
| Gastric cancer                             | Vit E, $\beta$ -carotene, selenium   |   | ↓                                     |
| Lung cancer in smokers                     | Vit E, $\beta$ -carotene and both together   |   | ↔                                     |
| Prostate cancer                            | Vit E  |   | ↓                                     |
| Lung cancer in workers exposed to asbestos | $\beta$ -carotene + Vit A  |   | ↔                                     |
| Lung cancer                                | $\alpha$ , $\beta$ -carotene, lutein, lycopene and $\beta$ -crypto-xanthine in diet for 10 years |   | ↓ In non-smokers                      |
| <i>Cardiovascular diseases</i>             |  |   |                                       |
| Myocardial infarction                      | Aspirin  |   | ↓                                     |
|  | $\beta$ -carotene  |   | ↔                                     |
| Coronary heart disease                     | Vit E  |   | ↓                                     |
| Atherosclerosis                            | Vit E  | Stenosis  | ↓                                     |
| Stroke and myocardial infarction           | N-3 PUFA , Vit E And both together   |   | ↓                                     |
| Cardiovascular disease                     | Catechin, Quercetin  | Collagen-induced platelet aggregation                                     | ↓                                     |
| Coronary heart disease                     | Vit C  | Improved vascular flow  | ↓                                     |
| Hypertension                               | Vit C  |   | ↓                                     |
| Heart failure                              | Carvedilol (25 mg bid) Metoprolol (50 mg bid) for 4, 8, 12 weeks.                                | Peripheral vascular resistance  | ↓ heart rate and ↑ exercise tolerance |
| <i>Neurodegenerative diseases</i>          |  |   |                                       |
| Parkinson's disease                        | Vit E. (2000 UI/day), Deprenyl (10 mg/day) and in combination for 14 months                      |   |                                       |
| Alzheimer's disease                        | Selegiline(10mg/day), Vit E (2000 UI/day) and in combination for 2 yrs                           | Death, institutionalization, severe dementia                              | ↓                                     |
| <i>Others</i>                              |  |   |                                       |
| Diabetes/hyperglycemia                     | Vit C (24 mg/min) intra arterially for 10 min  | Blood flow, Impaired endothelium-dependent vasodilatation                 | Restores                              |
| Type 2 diabetes                            | Vit E  | Age, body-mass index, fasting plasma glucose and insulin, vit E and TBARS | ↔                                     |
| Renal dysfunction                          | Acetylcysteine (600 mg bid) i.v.   | Serum creatinine and blood urea nitrogen levels                           | ↓                                     |
| Subarachnoid hemorrhage in mice            | Transgenic Cu-Zn SOD (22.7 U/mg protein) as compared to 7.9 in non transgenic mice               | iNOS levels   | ↓ levels of iNOS                      |
| Pre-eclampsia                              | Vit C (1000 mg) + Vit E (400 mg) during pregnancy  | Plasminogen activator inhibitor (PA-I), a marker                          | ↓ in PA-I                             |

↓ reduced effect; ↔ no significant effect; ↑ increased effect

(Heart-leaved moonseed, Guduchi), *Trigonella foenum-graecum* (Fenugreek), *Withania somnifera* (Winter cherry, Ashwagandha) and *Zingiber officinalis* (Ginger). There are also a number of ayurvedic formulations containing ingredients from medicinal plants that show antioxidant activities (Tilak *et al*, 2001).<sup>20</sup>

In respect of the above we would like to give some data on our studies with *Terminalia arjuna* (Tilak *et al*, in press).<sup>21</sup> Since possible antioxidant properties have been correlated with cardioprotective effects (Yoshikawa *et al*),<sup>4</sup> we examined the antioxidant properties of different preparations from the bark of *T. arjuna* that has been extensively studied for its cardioprotective effects (Tilak *et al*, in press-b).<sup>21</sup> One of its active ingredient, baicalein, also was subjected to these studies. The assays employed pertain to different levels of antioxidant protection such as radical formation, radical scavenging and membrane damage. Our studies have shown that the extracts of *T. arjuna* bark and baicalein possessed significant antioxidant properties. We also studied the possible uptake of components from *T. arjuna* and baicalain

by feeding rats and by using the ex vivo model of 'inverted rat intestine loop'. Our studies indicate that about 25% baicalein is taken up and other components are being absorbed. These components may confer protective properties by their antioxidant effects. Further studies are needed to characterize and to estimate their metabolism of such potentially useful components.

#### **Importance of phytonutrients**

The idea of growing crops for health rather than for food or fiber is slowly changing plant biotechnology and medicine. Rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multicomponent botanical drugs, dietary supplements, functional foods and plant-produced recombinant proteins. Among polyphenols, flavonoids constitute the most important single group, including more than 5000 compounds that have been thus far identified.

Apart from nutrient components such as  $\beta$ -carotene, vitamins C and E, and selenium, compounds such as phenols,

**Table 3 : Major group of antioxidant compounds and their dietary sources**

| Polyphenolic compounds   | Dietary sources   |
|--|---|
| <p>Flavonoids with antioxidant effect:<br/>                     Anthocyanidins, Aurones, Chalcones, Flavanones (Naringenin),<br/>                     Flavanols (Procyanidin), Flavan-3-ol (Epicatechin, Catechin),<br/>                     Flavones (Apigenin, Luteolin), Flavonols (Isorhamnetin, Kaempferol,<br/>                     Myricetin, Quercetin, Quercetin glycosides, Rutin), Isoflavonoids<br/>                     (Anisole, Cumestrol, Daidzein, Genistein)<br/>                     Other Polyphenols:<br/>                     Cinnamic acid, Coumarin, Condensed tannins, Hydroxybenzoic acid<br/>                     (Gallic acid, Protocatechuic acid, Vanillic acid), Hydroxycinnamic acid<br/>                     (Caffeic acid, Caftaric acid, Chlorogenic acid, Coumaric acid,<br/>                     , Ferulic acid, Sinapic acid), Proanthocyanidins,<br/>                     Carotenoids with antioxidant effects: Astaxanthin, Bixin,<br/>                     Canthaxanthin, Capsorubin, <math>\alpha</math>-Carotene, <math>\beta</math>-Carotene, <math>\gamma</math>-Carotene,<br/>                     Crocin, <math>\beta</math>-Cryptoxanthin, Lutein, Lycopene, Zeaxanthin,</p> | <p>Fruits: Apples, Blackberries, Blueberries, Citrus fruits, Grapes, Pears,<br/>                     Pomegranate, Raspberries, Strawberries<br/>                     Vegetables: Beetroot, Brinjal, Broccoli, Celery, Endives, Leek, Lettuce,<br/>                     Onion (white and red), Pepper, Spinach, Tomatoes<br/>                     Legumes: Horsegram, Greengram, Lupin Peas, Soy beans, White and<br/>                     Black Beans Spices: Cardamom, Cinnamon, Cloves, Coriander, Cumin<br/>                     Beverages: Cocoa, Tea, Wine (Red and White Wines, Sherry)<br/>                     Oil:Olive oil<br/>                     Chocolates</p>  |
| <p>Vitamins: Vitamin C, Vitamin E (<math>\alpha</math> Tocopherols, Tocotrienols),<br/>                     Nicotinamide.</p>  | <p>Fruits: Apples, Apricot, Banana, Blackberries, Blueberries, Cherries,<br/>                     Grapefruits, Grapes, Jack fruit, Kiwi fruit, Lemon, Mango, Melon,<br/>                     Orange, Papaya, Peach, Pears, Pineapple, Plum, Strawberries,<br/>                     Watermelon.<br/>                     Vegetables: Amaranthus, Asparagus, Beet Beetroot, Brinjal, Broccoli,<br/>                     Brussels sprouts, Cabbage, Cauliflower, Cucumber Carrots, Celery,<br/>                     Lettuce, Mushroom, Onion Pepper, Tomatoes, Potatoes, Pumpkin,<br/>                     Spinach, Spring greens, Spring onions.<br/>                     Cereals: Sweetcorn/Corn.<br/>                     Legumes: Beans (Broad, Green, Runner, Kidney), Bean sprouts, Peas.<br/>                     Spices: Chillies, Saffron<br/>                     Oil: Red palm oil<br/>                     Dairy products: Butter, Cheese, Margarine, Milk, Yogurt.<br/>                     Eggs: Whole and yolk, Mayonnaise<br/>                     Amla (Indian gooseberry), Lemon, Oranges,<br/>                     Oil: Groundnut oil, Olive oil, Palm oil, Cashew nuts, Germinated pulses,<br/>                     Rasins</p> |
| <p>Other compounds: Curcumin, Caffeine, Chlorophyllin, Sesaminol,<br/>                     Zingerone<br/>                     Functional Foods</p>   | <p>Coffee, Cocoa, Colas, Green vegetables, Tea, Turmeric, Zinger,<br/>                     Food colorants<br/>                     Soy protein, fish oil fatty acids, bengal gram, amla, bitter gourd, til,<br/>                     winged bean, guar, Spirulina (blue green algae), sunflower, cowpea,<br/>                     linseed, ground nut, kenaf, safflower, rape seed, horse gram, rice bran,<br/>                     pearl millet, wheat grass, sorghum, soybean, Amaranthus, ivy guard,<br/>                     cabbage, cassava, sweet potato and yams</p>  |

flavonoids, isoflavones, isothiocyanates, diterpenes, methylxanthines, dithiols, and coumarins appear to be important in cancer prevention through their role on the inhibition of tumor production. (Krishnaswami, 1996).<sup>22</sup>

### Functional foods

The very concept of food is changing from a past emphasis on health maintenance to the promising use of foods to promote better health to prevent chronic illnesses. ‘Functional foods’ are those that provide more than simple nutrition; they supply additional physiological benefit to the consumer. Because dietary habits are specific to populations and vary widely, it is necessary to study the disease-preventive potential of functional micronutrients in the regional diets. Indian food constituents such as spices as well as medicinal plants with increased levels of essential vitamins and nutrients (eg. vitamin E, lycopene, vitamin C, bioflavonoids, thioredoxin etc.) provide a rich source of compounds like antioxidants that can be used in functional foods (Devasagayam *et al.* In Press).<sup>23</sup>

### Pro-oxidant effect of antioxidant under certain conditions

Antioxidants also have the potential to act as prooxidants

under certain conditions. For example, ascorbate, in the presence of high concentration of ferric iron, is a potent potentiator of lipid peroxidation. Recent studies suggest that ascorbate sometimes increase DNA damage in humans. Recent mechanistic studies on the early stage of LDL oxidation show that the role of vitamin E is not simply that of a classical antioxidant. Unless additional compounds are present, vitamin E can have antioxidant, neutral or prooxidant activity. Beta-carotene also can behave as a prooxidant in the lungs of smokers.

The paradoxical role (pro-oxidant effect) of antioxidants is also directly related to the recently described ‘redox signaling’ of the antioxidants. The functional role of many antioxidants depends on redox cycling. For example, the best-described intracellular antioxidant vitamin E supplementation in the face of infarcted myocardium exerted prooxidant effects resulting in the rupture of the plaques. When a cell is attacked by environmental stress, the cell’s defense is lowered because of massive generation of ROS. The cell immediately responds to this stress by upregulating its antioxidant defense. During the induction process ROS function as signaling molecules. It should be easily understood that in these pathophysiological conditions even though the antioxidants



## Neurodegenerative Disorders

Nervous tissue including brain is highly susceptible for free radical damage due to high content of lipids especially polyunsaturated fatty acids. In Alzheimer's disease (AD) biochemical and histological studies have provided evidence for increased levels of oxidative stress and membrane LP. Alterations in levels of antioxidant enzymes such as catalase and CuZn- and Mn-SOD in neurons in AD patients are consistent with their being under increased stress. Increased protein oxidation, protein nitration and LP occur in neurofibrillary tangles and neuritic plaques. Lipid peroxidation is quite extensive as indicated by increased levels of peroxidation products such as 4-hydroxynonenal (4-HNE) in the cerebrospinal fluid of AD patients. Iron ( $\text{Fe}^{2+}$ ) likely contributes to increased LP in AD. Lipid peroxidation may promote neuronal death in AD by multiple mechanisms that include impairment of the function of membrane ion-motive ATPases ( $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{Ca}^{2+}$ -ATPase), glucose transporters and glutamate transporters. Lipid peroxidation leads to production of the aldehyde 4-HNE that appears to play a central role in the neurotoxic actions of amyloid  $\beta$ -peptide (Yoshikawa *et al.* 2000).<sup>4</sup>

## Free Radical Damage to DNA and Cancer

DNA is a major target of free radical damage. The types of damages induced are many and include strand breaks (single or double strand breaks), various forms of base damage yielding products such as 8-hydroxyguanosine, thymine glycol or abasic sites, damage to deoxyribose sugar as well as DNA protein cross links. These damages can result in mutations that are heritable change in the DNA that can yield cancer in somatic cells or foetal malformations in the germ cells. The involvement of free radicals with tumor suppressor genes and proto-oncogenes suggest their role in the development of different human cancers (Halliwell and Aruoma, 1993).<sup>11</sup> Cancer develops through an accumulation of genetic changes. Initiating agents can be tobacco smoking and chewing, UV rays of sunlight, radiation, viruses, chemical pollutants, etc. Promoting agents include hormones (androgens for prostate cancer, estrogens for breast cancer and ovarian cancer). Inflammation induces iNOS (inducible nitric oxide synthase) as well as COX and LOX. These can initiate carcinogenesis.

Experimental as well as epidemiological data indicate that a variety of nutritional factors can act as antioxidants and inhibit the process of cancer development and reduce cancer risk. Some of these include vitamins A, C, E, beta-carotene, and micronutrients such as antioxidants and anticarcinogens. (Croce, 2001).<sup>27</sup> Surh (2003)<sup>28</sup> recently reviewed mechanisms behind anticancer effects of dietary phytochemicals. Chemopreventive phytochemicals can block initiation or reverse the promotion stage of multistep carcinogenesis. They can also halt or retard the progression of precancerous cells into the malignant ones. Many molecular alterations associated with carcinogenesis occur in cell-signalling pathways that regulate cell proliferation and differentiation. One of the central components of the intracellular- signalling

network that maintains homeostasis is the family of mitogen-activated protein kinases (MAPKs). Numerous intracellular signal-transduction pathways converge with the activation of the transcription factors NF- $\kappa$ B and AP1. As these factors mediate pleiotropic effects of both external and internal stimuli in the cellular- signalling cascades, they are prime targets of diverse classes of chemopreventive phytochemicals (Surh, 2003).<sup>28</sup>

The active principle of *Curcuma longa* (Turmeric, Haldi) - curcumin, down-regulates the expression of COX2, LOX, iNOS, MMP-9, TNF, chemokines and other cell-surface adhesion molecules and cyclin D1. Human clinical trials have shown safety at doses upto 10 g/day curcumin, which can suppress tumour initiation, promotion and metastasis. Many Ayurvedic herbal drugs have anti-inflammatory, antioxidant and immunomodulatory activity and can be used for chemoprevention. Validation of the concept necessitates long-term prospective clinical studies.

## Free Radicals, Diabetes and AGEs

Experimental evidences suggest the involvement of free radicals in the onset of diabetes and more importantly in the development of diabetic complications (Lipinsky, 2001).<sup>29</sup> Scavengers of free radicals are effective in preventing experimental diabetes in animal models and in type 1 (IDDM) and type 2 (NIDDM) patients as well as reducing severity of diabetic complications. Persistent hyperglycemia in the diabetic patients leads to generation of oxidative stress due to a) autooxidation of glucose; b) non-enzymatic glycosylation and c) polyol pathway. Auto-oxidation of glucose involves spontaneous reduction of molecular oxygen to superoxide and hydroxyl radicals, which are highly reactive and interact with all biomolecules. They also accelerate formation of advanced glycation end products (AGEs). AGEs such as pyrroles and imidazoles tend to accumulate in the tissue. Crosslinking AGE-protein with other macromolecules in tissues results in abnormalities in the cell and tissue function. Polyol pathway is the third mechanism by which free radicals are generated in the tissues (Glugliano *et al.*, 1995).<sup>30</sup> Lot of NADPH is deleted during this pathway, hence it impairs generation of antioxidants such as glutathione. Due to protein glycation capacity of antioxidant enzymes is also reduced. Free radicals generated also react with nitric oxide in endothelial cells leading to loss of vasodilation activity. Long-lived structural proteins, collagen and elastin, undergo continual non-enzymatic crosslinking during ageing and in diabetic individuals (Vasan *et al.* 2003).<sup>31</sup> This abnormal protein crosslinking is mediated by AGEs generated by non-enzymatic glycosylation of proteins by glucose.

## Free radicals and ageing

Mitochondrial ROS production and oxidative damage to mitochondrial DNA results in ageing. Further increased lipid peroxidation in cellular membranes due to oxidative stress leads to fatty acid unsaturation. The most recent review on 'free radicals and ageing' by Barja (2004)<sup>32</sup> emphasizes that caloric restriction (CR) is the only known experimental manipulation that decreases rate of mammalian ageing, and it

has many beneficial effects on the brains of rodents and possibly of humans. Calorie-restricted mitochondria, similar to those of long-lived animal species, avoid generation of ROS efficiently at complex I with pyruvate and malate. The mitochondrial oxygen consumption remains unchanged, but the free radical leak from electron transport chain is decreased in CR.

Many investigators realized that increasing the level of defense mechanisms against oxidative stress could extend an organism's health span. Arking's research group's work on artificial selection in flies also produced organisms with a much higher level of oxidative stress resistance and more efficient mitochondria. In fact, the lower level of oxidative damage and delayed onset of senescence in those flies arose from decreased production and increased destruction of ROS. However, using genetic engineering techniques to insert some extra copies of these oxidative stress-resistance genes into mice has not yet resulted in extending longevity (Lane *et al* 2002).<sup>33</sup>

#### **Mitochondria, oxidative protein damage and proteomics**

The rapid advance of proteomic methodologies and their application to large scale studies of protein-protein interactions and protein expression profiles suggest that these methods are well suited to provide the molecular details needed to fully understand oxidative injury induced by free radicals (Gibson, 2004).<sup>34</sup> Over the last two decades, considerable progress has been made in identifying individual proteins that are localized to the mitochondria. In particular the 100 or so subunits that constitute the five complexes of the electron transport chain (ETC).

Recently, using modern mass spectrometry (MS)-based proteomic strategies, several groups have begun to tackle the larger job of determining the composition of entire mitochondrial proteomes from a number of important model systems as well as from human tissues. Using mitochondria isolated from human heart, Gibson and coworkers have identified 684 unique proteins from the combined peptide data obtained from over 100,000 mass spectra generated by MALDI-MS and high performance liquid chromatography (HPLC) MS/MS analyses. These data are now part of 'MitoProteome', a publicly accessible database for the human heart mitochondrial proteome.

### **NEWER AND NOVEL APPROACHES TO REDUCE FREE RADICAL DAMAGE AND FUTURE PROSPECTS**

There are several novel approaches in the study of free radicals/antioxidants for the improvement of human health. A number of neuronal and behavioral changes occur with ageing, even in the absence of degenerative disease. A decline in cognitive function is one of the manifestations of changes that occur in neuronal functions with age. Several recent studies have found associations between the decline of memory performance and lower status of dietary antioxidants. The totality of evidence from experimental, clinical, and

epidemiological studies support the notion that consumption of foods obtaining high levels of dietary antioxidants, in addition to exerting several health benefits, may prevent or reduce the risk of cognitive deterioration.

Recently a new class of SOD mimetic drugs, like tempol, was also developed to alleviate acute and chronic pain. These drugs substantially reduced tissue damage by inflammation and reperfusion. Unlike the naturally derived SOD enzymes the mimetic is well suited for use as a drug because it has a much lower molecular weight, is more stable and appears not to elicit an immune response in the body. SOD mimics also have ability to increase antitumour effects of interleukins, besides being efficient radioprotectors.

Development of genetically engineered plants, to yield vegetables with higher level of certain compounds is another approach to increase antioxidant availability. Tomatoes with upto 3 times lycopene concentration as well as with longer shelf life were developed. 'Orange cauliflower' is found to be rich in (-carotene. One way of checking the antioxidant ability of vegetables and fruits is measuring its ORAC value or oxygen radical absorbance capacity. Some fruits/vegetables with their ORAC values/100 g in (brackets) are raisins (2830), black berries (2036), strawberries (1540), oranges (750), grapes (739), cherries (670), spinach (1260), beets (840), onion (450) and eggplant (390). Intake of fruits and vegetables with ORAC values between 3000 and 5000 per day is recommended to have significant impact of the beneficial effect of antioxidants (Lachnicht, 2000).<sup>35</sup>

Given their inherently nutritious composition, most fruit and vegetable breeding programmes have tended to focus on improving aspects like appearance, taste, texture, shape and shelf-life. But more and more research energy is being channeled into making fruits and vegetables even healthier. The Vegetable and Fruit Improvement Cente at Texas A and M University is a leading research hub in the area and created one of the world's first 'super-vegetables' about a decade ago - a purple carrot breed that had 40% more beta-carotene than usual. The carrot, 'Betasweet', now available throughout the US, was also bred to have a higher sugar content to improve flavour, as well as a crispy texture to make it more palatable for children. Other similar work being conducted include: developing the anti-carcinogen properties of citrus fruits; increasing the carotenoid content of watermelons and cantaloupes; developing milder and sweeter onions with increased quercetin and anthocyanin levels; increasing the quercetin levels of peppers and making them sweeter; increasing anthocyanin levels in stone fruits; developing lycopene-enriched tomatoes.

Among the different products delivering essential nutrients to the body, an egg arguably has a special place, being a rich and balanced source of essential amino and fatty acids as well some minerals and vitamins. The advantages of simultaneous enrichment of eggs with vitamin E, carotenoids, selenium and DHA include better stability of polyunsaturated fatty acids during egg storage and cooking, high availability of such nutrients as vitamin E and carotenoids, absence of

off-taste and an improved anti-oxidant and n-3status of people consuming these eggs. Designer eggs can be considered as a new type of functional food. Indian traditional systems of medicine and home remedies have identified many forms of health foods. Mainly whole foods are consumed as functional foods rather than supplements or processed foods.

It is a time, after a period of flouting research on oxidants and antioxidants, to critically reflect on the fields. Speculation that many (if not all) diseases are related to radical damage needs to be supported by more secure data. The hope that antioxidants can prevent or cure a number of pathological situations also required reconsideration. The relatively new notion that molecules with strong antioxidant activity in vitro may have 'non-antioxidant' effects in cells and tissues should stimulate, rather than discourage, important research in this field. Finally, the discrepancies in the outcome of intervention studies may be understood if, instead of considering the simple paradigm of bad oxidants and good antioxidants, scientists will start talk about the real molecular function of such compounds in each particular situation (Azzi *et al.*, 2004).<sup>36</sup>

## 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 around 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. Co-ordinated 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.

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