Antioxidants: New-generation therapeutic base for treatment of polygenic disorders

Ashok K. Tiwari

Pharmacology Division, Indian Institute of Chemical Technology, Hyderabad 500 007, India

Hyperphysiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's disease and Parkinsonism. Therefore, in modern Western medicine, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system. Researches in the recent past have accumulated enormous evidence advocating enrichment of body systems with antioxidants to correct vitiated homeostasis and prevent the onset as well as treat the disease caused/fostered due to free radicals and related oxidative stress. 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.

ANTOINE Lavoisier, a pioneer oxygen chemist, had pointed out about 150 years ago that animals that respire are true combustible bodies that burn and consume themselves[1]. The biological combustion produces harmful intermediates called free radicals. A free-radical is simply defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electrons being one that is alone in an orbital. It may be superoxide ($O_2^{\bullet-}$, an oxygen centred radical), thiyl ($RS^{\bullet}$, a sulphur-centred radical), trichloromethyl ($CCl_3^{\bullet}$, a carbon centred radical) or nitric oxide ($NO^{\bullet}$) in which the unpaired electron is delocalized between both atoms. The $O_2^{\bullet-}$, hydroxyl radicals ($^{\bullet}OH$) and other reactive oxygen species (ROS) such as H_2O_2 are continuously produced *in vivo*.

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated immune functions. These free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants, pesticides, etc.[2]. These exogenous pollutants generating free radicals, have become part and parcel of our daily inhaling/ingesting life and infact there appears no escape from them. Continuous interaction of the animal physiological systems with these free radicals generated either indigenously or inhaled/ingested from exogenous sources therefore, lead to excess load of free radicals and cause cumulative damage of protein, lipid, DNA, carbohydrates and membrane, resulting in so-called oxidative stress. Therefore, living creatures have evolved a highly complicated defence system with antioxidants composed of enzymes and vitamins against oxidative stress in the course of their evolution. These defence systems are mainly classified[3] as (i) suppression of generation of ROS, (ii) scavenging of ROS, (iii) clearance, repairing and reconstitution of damage and (iv) induction of antioxidant proteins and enzymes.

However, amounts of these protective devices present under normal physiological conditions are sufficient only to cope with the normal threshold of physiological rate of free-radical generation. Therefore, any additional burden of free radicals, either from an indigenous or exogenous source on the animal (human) physiological system can tip free radical (prooxidant) and anti-free radical (antioxidant) balance leading to oxidative stress[2]. The oxidative stress, defined as the imbalance between oxidants and antioxidants in favour of the former potentially leading to damage has been suggested to be the cause of aging and various human diseases[4] (Figure 1). Therefore, in modern Western medicine, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system[5,6]. Any vitiation therefore, is understood to give rise to disorderliness in the physiological system leading to a variety of diseases depending upon the sensitivity and susceptibility of the organ. Thus, the status of protective mechanism against oxidants, the antioxidants in humans reflect the dynamic balance between antioxidant defence and prooxidant conditions and have been suggested as a useful tool in estimating the risk of oxidative damage[2,7–9].

Research in the recent past has accumulated enormous evidences revealing that enrichment of body systems with natural antioxidants may correct the vitiated homeostasis[10–13] and can prevent the onset as well as treat diseases caused and/or fostered due to free-radical mediated oxi-

This article is dedicated in the honour of Dr K. V. Raghavan, Ex-Director, IICT on the eve of his 60th birthday.

e-mail: tiwari@iict.ap.nic.in

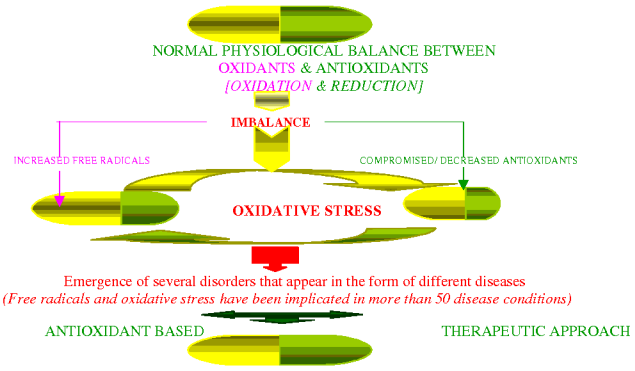

Figure 1. Oxidative stress occurs due either to the increased generation of free radicals or compromised and/or decreased antioxidant defence. This imbalance can be managed by exogenous supply of antioxidant rich nutrition, natural and/or synthetic antioxidant principles-based therapeutic preparations.

dative stress. These developments accelerated the search for antioxidant principles that lead to the identification of natural resources, isolation of active principles and further modification and refinement of active antioxidant molecules. However, animal experimentations and clinical evaluations with these active ingredients were not only punctuated by some success, but also by some spectacular failures[2]. Nevertheless, success and failures with these efforts and concurrent advancement in understanding of free-radical biology and related pathogenesis provided important insights in further improving the development of newer molecules/molecular compositions that may provide better preventive and therapeutic potentials. The perpetual tendency of learning towards refinement of understanding and the knowledge, has now led to the discovery and development of several novel antioxidant based molecules/drugs/natural medicinal compositions that have satisfied majority of the aspects of complex pathogenic steps in diseases for which only disease/risk factor-modifying therapies are available.

This article presents a general account on understanding the role of oxidative stress and efforts made in developing novel antioxidant-based drugs/formulations for prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, Parkinson's disease, cancer, etc. and discusses the scope for further strategic development of newer therapies.

Antioxidants

In foods, antioxidants have been defined as a substance that in small quantities is able to prevent or greatly retard the oxidation of easily oxidizable materials such as fats[14]. However, in biological systems the definition for antioxidants has been extended to any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate like lipids, proteins, DNA, and carbohydrates[15]. Currently however, biological antioxidants have further assumed a broad definition to include repair systems such as iron transport proteins (e.g. transferrin, albumin, ferritin and caeruloplasmin), antioxidant enzymes, and factors affecting vascular homeostasis, signal transduction and gene expression[16].

Antioxidants may exert their effects by different mechanisms, such as suppressing the formation of active species by reducing hydroperoxides (ROO$^\bullet$) and H_2O_2 and also by sequestering metal ions, scavenging active free radicals, repairing and/or clearing damage. Similarly, some antioxidants also induce the biosynthesis of other antioxidants or defence enzymes. The bioactivity of an antioxidant is dependent on several factors like their structural criteria, physico-chemical characteristics and *in vivo* radical generating conditions (see ref. 2 and references cited therein).

An antioxidant works by retarding the oxidation. In biology, oxidation is often started by free radicals. The role of an antioxidant is to intercept a free radical before it can react with the substrate. For example, phenol (AOH), the reaction of interest with ROO$^\bullet$ is:

$$AOH + ROO^\bullet \rightarrow AO^\bullet + ROOH.$$

This H-atom transfer reaction effectively stops chain reaction. Therefore, antioxidants of biological/therapeutic importance should have the property that they will react/trap the free-radical before it reacts with the susceptible substrate and initiate chain reaction. Based on several theoretical models and complex calculations, Wright[17] concluded that bond dissociation enthalpy (BDE) gives excellent correlation for this requirement with many known families of antioxidants that have been extensively studied in biological systems, like vitamins E and C, resveratrol, gallocatechins, ubiquinol, etc. He suggested that lower the BDE, the more reactive the antioxidant. However, it should not be too low to reduce the molecular oxygen, forming $HO_2^\bullet$ the process of autoxidation[17]. Major understanding of beneficial therapeutic activities of antioxidants has arisen with studies on vitamins E and C and ubiquinol Q_{10} that serve as excellent reference material. Mechanisms of radical scavenging activity of antioxidants and their pros and cons are well available[2,10]. The most active component of vitamin E, α-tocopherol, is a good lipid-soluble, chain-breaking antioxidant that breaks the cycle of lipid peroxidation by reacting with ROO$^\bullet$ before it can attack lipid molecules. Another excellent antioxidant is water-soluble vitamin C. Vitamin C is used to repair vitamin E radical[10]. Based on BDE calculations of these important antioxidants, Wright[17] proposed a design window for an antioxidant to be ideal. The useful design window for an ideal antioxidant based on BDE calculations may be in the range of 68–76 kcal mol^{-1}, i.e. higher than vitamin C and lower than α-tocopherol. Based on

these considerations, it has been made possible to design several antioxidant molecules that have displayed excellent biological activities. Simultaneously, it was also observed that majority of antioxidants originating from natural products fall under this criterion[17]. This understanding may provide future directions for design, synthesis and also search, identify and prioritize novel antioxidants from natural resources.

Progress in sciences is providing crucial insights in the understanding mechanisms of disease pathogenesis and is opening up rich field of potential targets for pharmaceutical intervention. The broader and clearer understanding of the molecular basis of disease processes therefore is paving a way to develop more effective and targetted treatment. 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 (ref. 18 and references cited therein). These wide varieties of chronic inflammatory diseases form the basis for development of antioxidant-based therapeutics. Regardless of their initiating pathological events, these diseases share a series of steps that lead to a common mechanistic pathway of oxidative stress through regulatory oxidative signals[19] (Figure 2). Various drug-discovery groups and programmes the world

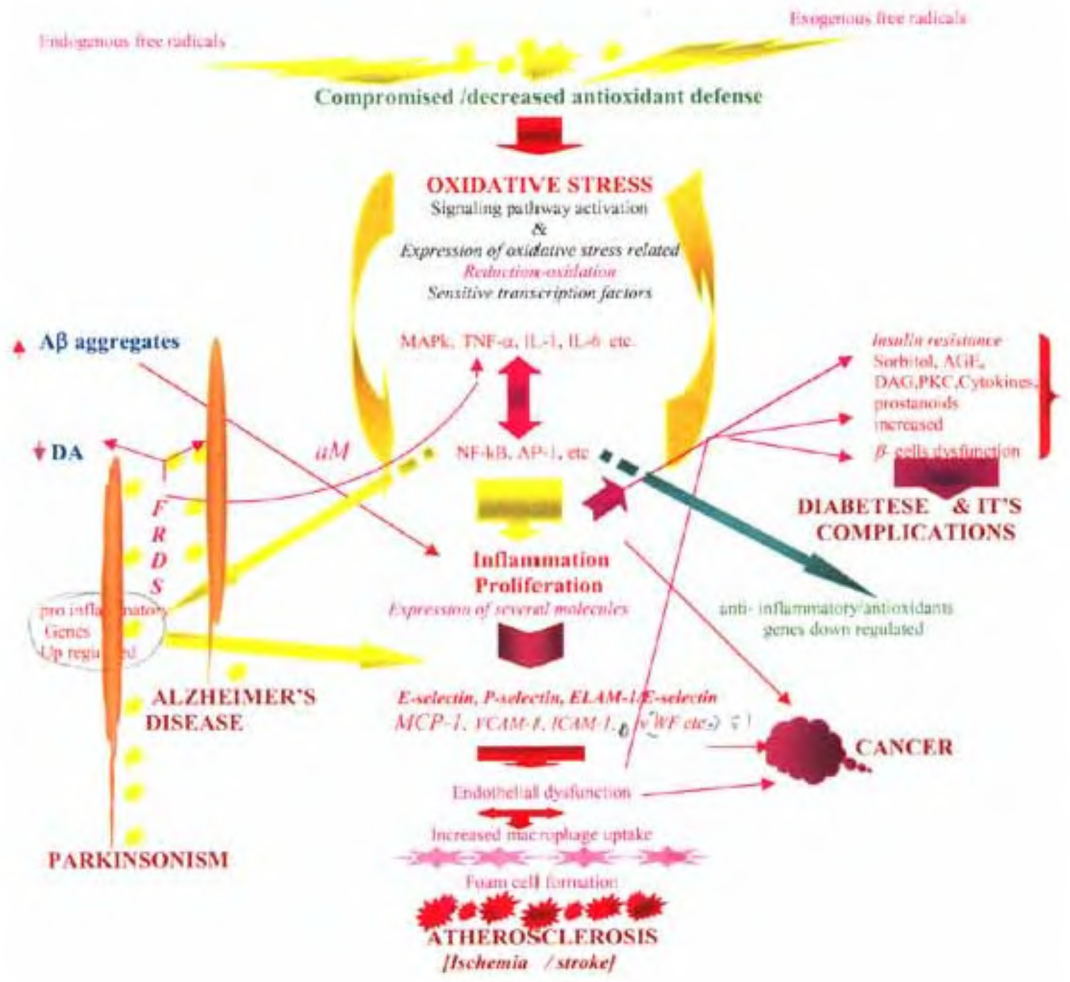

Figure 2. Involvement of free radicals and oxidative stress-induced expression of *red-ox* sensitive factors, cytokines and adhesion molecules leading to the development of various diseases. Free radicals act as signalling intermediate and initiate receptor-mediated activation of intracellular signalling pathways that activate the production of inflammatory chemokines and cytokines. MAPk cascades are activated by various free radicals, cellular stressors, and growth factors and are involved in several biological responses like cytokines production, differentiation, proliferation and cell death. TNF exerts a variety of biological effects like production of inflammatory cytokines, up-regulation of adhesion molecules, proliferation, differentiation and cell death. It induces free radicals accumulation and also acts as a strong activator of NF-kB. NF-kB is a transcriptional factor that regulates expression of various inflammatory cytokines, chemokines and adhesion molecules, and plays an important role in vascular cell functions. Aβ, Amyloid beta protein; AGE, Advanced glycation end-products; aM, Activated microglia; AP, Activator protein; DA, Dopamine; DAG, Diacylglycerol; ELAM, Endothelial leukocyte-adhesion molecule; FRDS, Free radicals; ICAM, Intracellular-adhesion molecule; IL, Interleukin; MAPk, Mitogen-activated protein kinase; MCP-1, Monocyte chemoattractant protein; NF-kB, Nuclear factor-kappa B; PKC, Protein kinase C; TNF, Tumour necrosis factor; VCAM, Vascular cell-adhesion molecule; vWF, von Willebrand factor. Details in the text and references cited appropriately at respective places.

over have excelled in designing and developing novel antioxidant-based drug molecules that have proven their therapeutic efficacy and have gathered information for further advancements in designing and developing drug molecules for treatment of diseases where no satisfactory therapy is available.

Atherosclerosis

Oxidative stress, especially oxidation of low-density lipoproteins (LDL), has long been suspected to play a critical role in atherogenesis, in consequence of which antioxidants were expected to have antiatherogenic potential. Such agents were thought to be able to inhibit oxidative modification of LDL that leads to the accumulation of cholesterol in the atherosclerotic lesion[20–22].

Furthermore, insights gained from genomic analysis of both the oxidized lipids and antioxidants have further changed the perspective of the etiology of this complex disease. The findings by genomic analysis provide key elements for molecular mechanisms that contribute to the antiatherosclerotic properties of phenolic antioxidants. Differences in gene expression in various animal models and in different regions of the aorta in response to these antioxidants may be one reason that antiatherogenic effects vary greatly depending upon the animal model and site of disease progression[23]. It was observed that high fat diet increased 32 genes; however, only three genes were observed to be related to atherogenesis. On the other hand, some of the genes showing decreased expression were those of free-radical scavenging enzymes, resulting in increase of oxidative stress, which was attenuated by antioxidant supplementation[24]. These observations support the view that hyperlipidemia is merely a risk factor and oxidative stress is the *root cause* of atherogenesis.

Emerging evidence therefore, for diverse functions of both the phenolic antioxidants and oxidized lipids at molecular levels, clearly guides for development of global systems approach. However, for those actively engaged in developing potential therapeutics, clinical trials of antioxidants have given rise to some controversies regarding their real clinical benefits[25–27]. Though several compounds classified as antioxidants have been shown to have antioxidant properties, probucol[23] and vitamin E as α-tocopherol[28] happened to be the pioneer antioxidant candidates that have made basis for the understanding of their mode of action and provided substantial insights in further designing and developing novel antioxidant-based molecules, encompassing important structural/functional features to impart better therapeutic potential and also overcome the unwanted effects.

Many therapeutic agents have been developed to counteract major risk factors for cardiovascular disease like hyperlipidemia and hypertension. However, no therapy is available to address the root cause of atherosclerosis. Recently, two groups have emerged with success in designing, developing and taking antioxidant-based antiatherosclerotic candidates to the level of clinical-trials[28,29]. The ultimate clinical success of these candidates (Figure 3) will no doubt revolutionize the therapy of atherosclerotic diseases. Similarly, several therapeutic preparations from traditional medicines like *Terminalia arjuna*[30,31], Abana[32,33], Campo medicine[34], and MAK[35] may have significant therapeutic potential to address antiatherosclerotic properties in clinical settings.

Stroke

As recently as five years ago, most physicians would have confidently described atherosclerosis as a straight plumbing problem. In atherogenesis, fat-laden gunk gradually builds up on the surface of passive artery walls and when the deposit (plaque) grows large enough, it eventually closes off an affected 'pipe', preventing blood from reaching its intended tissue; after a while, the blood-starved tissue dies. As a result, when a part of the cardiac muscle succumbs, it leads to heart attack and when it affects the brain it is called a stroke. Many strokes stem instead from less obstructive plaques that rupture suddenly, triggering the emergence of a blood clot, or thrombus, that blocks blood flow[36].

Stroke is the third leading cause of death and the major cause of disability in USA. In the general population, incidence of stroke is 1/1000 individuals, however, incidence doubles in individuals who are 80 years of age[37]. Stroke is defined as an abrupt impairment of brain function resulting from occlusion or rupture of intra or extra cranial blood vessels. There are several types of stroke:

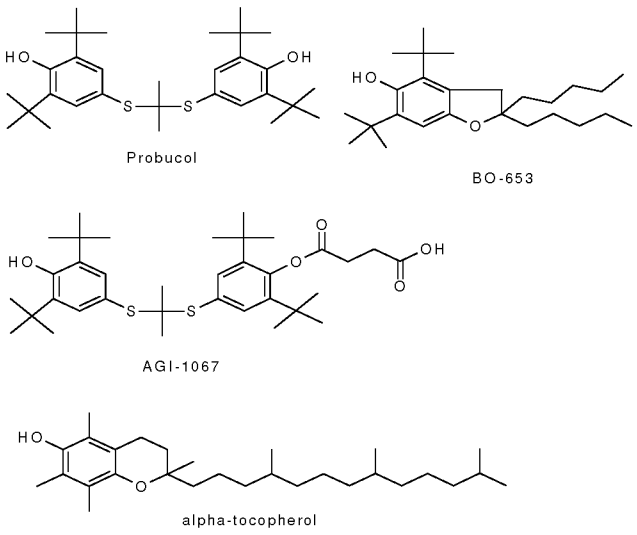

Figure 3. Structure of important compounds discussed in the literature. BO-653 and AGI-1067 are the recent developments up to clinical trial as antiatherosclerotic antioxidants. Probucol and alpha tocopherol serve as the basis for the design and development of these compounds.

cerebral thrombosis and cerebral embolism, also classified as ischaemic stroke and subarachnoid haemorrhage, and intracerebral haemorrhage also classified as haemorrhagic stroke.

Cerebral thrombosis is the most common type of stroke and occurs when a thrombus develops on the wall of a cerebral artery, usually damaged by atherosclerosis[37]. Therefore, therapeutics developed for atherosclerosis, based on imbalance between oxidant and antioxidant homeostasis appears to be important in treating stroke provided it reaches the brain-tissue sites. Extensive research generated in the recent past has disclosed that free radicals play a major role in the damage caused by hypoxia and reperfusion during cerebral ischaemia, affecting a late stage of the ischaemic process. These developments also support views that agents that scavenge free radicals or prevent their production may be able to prolong the therapeutic time window[37]. Several antioxidants and free-radical scavenging-based therapeutics have been recently launched and are under development for treatment of stroke[37].

Diabetes and diabetic complications

There is considerable evidence that hyperglycemia results in the generation of ROS, ultimately leading to increased oxidative stress in a variety of tissues. In the absence of an appropriate compensatory response from indigenous antioxidant network, the system becomes overwhelmed (redox imbalance), leading to the activation of stress-sensitive intracellular signalling pathways. One major consequence of this is the expression of gene products that cause cellular damage and are ultimately responsible for late diabetic complications (Figure 2). Apart from playing a key role in late diabetic complications, activation of it or similar signalling pathways also appears to play a role in mediating insulin resistance and impaired insulin secretion. The ability of antioxidant/free-radical scavengers to protect against the effects of hyperglycemia and free fatty acids along with clinical benefits following antioxidant therapy, supports the causative role of oxidative stress in mediating and/or worsening these abnormalities[38]. A number of reviews have appeared recently, stressing the role of oxidative stress in pathogenesis of cellular dysfunction leading to cardiovascular, hepatic and other complications of diabetes[38–40].

Similarly, supplementation with antioxidants has also been shown to decrease oxidative stress and complications in animal models of diabetes[41,42] and diabetic patients[43]. Diabetes-induced defects in the homeostasis and the transport of intracellular calcium have been shown to decrease or recover by treatment of diabetic animals with some antioxidants[44]. Several studies have demonstrated that antioxidants supplementation prevents lipid peroxidation, haemoglobin glycation and inhibition of Na^+, K^+-ATPase and/or Ca^{++}-ATPase activity caused by hyperglycemia in various cells[45,46].

Stobadine is a synthetic drug and scavenges a variety of free radicals (ref. 44 and references therein). Blood glucose-lowering effect of stobadine treatment in streptozotocine (STZ)-induced diabetes in animals starts two days after the STZ injection and its effect has been directly correlated to its free-radical scavenging properties, which may protect pancreatic β-cells against STZ toxicity[47]. Stobadine in low dose is able to lower blood glucose and tissue calcium accumulation in STZ-diabetic rat. It has also been observed that together with vitamin E, it can provide better control on hyperglycemia-induced oxidative stress[44].

Multiple activities of phytochemicals present in traditional medicines and their preparations have been reviewed recently[48]. There are several medicinal plants the world over used in traditional medicine, which possess rich antioxidant principles and strong antioxidant activities. It has been argued that major antidiabetic activities from these plants might originate from their antioxidant principles[49,50]. Taking the advantage of modern drugs like stobadine and its detailed mechanism of action, natural medicines may also be developed explaining their therapeutic properties and mechanism of action. These efforts may provide novel mechanism-based application of traditional medicines used in this disorder.

Neurodegenerative diseases

Aging is the major risk factor for neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). Oxidative stress may induce neuronal damage, ultimately leading to neuronal death by apoptosis or necrosis. A large body of evidence indicates that oxidative stress is involved in the pathogenesis of AD and PD. Simultaneously, increasing number of studies show that nutritional antioxidants can block neuronal death and may have therapeutic properties in animal models of these neurodegenerative diseases[51,52].

Alzheimer's disease

The risk of acquiring AD is considerable in countries with long life expectancies. Projected national burdens related to AD are daunting as unprecedented numbers of people are expected to survive their eighth and ninth decade of life. In USA alone, the current estimate of 3.6 lakh new cases of AD each year is expected to triple in the next 40 years[53]. AD is the commonest form of dementia with a prevalence of 0.4% in women and 0.3% in men aged 60–69 years[54]. Estimated prevalence of senile dementia in Europe increases with age from 1% in man and women of age 60 years to 44.7% in a population of 90–95 years age[55].

Selective sensitivity and vulnerability of neurons are the most important characteristics of this disorder. Free-radical theory of aging suggests that oxidative damage is a major player in degeneration of cells[56]. The role of oxidative stress in the etiology of AD has long been hypothesized, described, and supported by a variety of experimental and clinical studies. This research has also promoted interest in assessing antioxidants for their possible benefits in modifying the course, reducing the risk, or delaying the onset of AD. Recent research reveals that dietary antioxidants may have promising therapeutic potential in delaying the onset as well as preventing the aging population with AD and its related complications[57]. Characteristic histopathological alterations in AD are neutristic plaques composed largely of amyloid β-peptides (Aβ) and neuronal aggregates of abnormally phosphorylated cyctoskeletal proteins[52]. Several lines of evidence (ref. 51 and references cited therein) suggest that over production of ROS is implicated in Aβ neurotoxicity: (i) exposure of cultured neurons or neuronal cell lines to Aβ increases intracellular levels of ROS leading to the activation of NF-kB; (ii) markers of oxidative stress are found increased in transgenic mouse models of AD; (iii) neurotoxicity of Aβ is attenuated by antioxidants such as vitamin E, PBN (α-phenyl-tert-butylnitrone), lazaroids and free-radical scavengers.

A controlled clinical trail with DL-α-tocopherol and selegiline in patients with moderately severe impairments of AD has shown some beneficial effects with respect to the rate of deterioration of cognitive function[58]. Vitamin E has been proposed to impart beneficial effect in this connection by quenching the ROS formed, and selegiline protects neurons by preventing the formation of ROS and by inhibiting oxidative metabolism of catecholamines. These advances provide a sound basis for search, design and development of targetted antioxidants for prevention and treatment of AD.

Parkinson's disease

PD is a neurological syndrome manifested by any combination of tremor at rest, rigidity, bradykinesia and loss of postural reflexes. Neuropathalogical hallmark of PD is selective degeneration of dopaminergic neurons in the nigrostriatal system[59]. These neurons synthesize and release dopamine (DA), and loss of dopaminergic influence on other structures in the basal ganglia leads to classical Parkinsionian symptoms[51].

Epidemiological studies indicate that a number of factors like exposure to herbicides, industrial chemicals, trace metals, cyanide, organic solvents, carbon monoxide and carbon disulphide may increase the risk of developing PD[60]. Majority of them are known to increase ROS and oxidative stress. Oxidative stress may arise from the metabolism of DA with the production of potentially harmful free radicals[61]. Alterations in pro- and antioxidant molecules have also been observed in post-mortem tissues from individuals with PD[51]. Activated microglia (aM) are thought to contribute to neuronal damage via the release of proinflammatory and neurotoxic factors like TNFα, IL-1, RNS, and ROS, etc. The reactive free radicals and their downstream products have been shown to contribute substantially to the oxidative damage in PD. Markers of elevated accumulation of NO, ROS, TNFα, IL-1β, INF-γ in substantia nigra of PD patients have been demonstrated[62].

Two neuroprotective clinical trials are available with antioxidants: (i) Deprenyl and tocopherol antioxidant therapy of Parkinson's study observed that deprenyl[63], an antioxidant molecule and also MAO-B inhibitor slowed early progression of symptoms and delayed the emergence of disability by an average of nine months. However, vitamin E at a given dose could not display significant effects. (ii) But in another open trial, combination of high dosage of α-tocopherol and ascorbate delayed the emergence of disability by 2.5 years, the time necessary to begin therapy with L-DOPA[64]. Esposito *et al.*[51] suggest that there are many alternative antioxidative approaches that may be considered in future clinical trials, including free radical scavengers, indigenous antioxidant enzyme boosters, iron chelators and drugs that interfere with oxidative metabolism of DA in Parkinsonism.

Cancer

The recent world cancer report released by WHO observes that world cancer rates are set to double by 2020[65]. Cancer is emerging as a major problem globally; both in more developed and in less developed countries. Furthermore, cancer mortality in the world as a whole is more than twice that in developing countries, a factor the report attributes to the earlier onset of the tobacco epidemic, earlier exposure to occupational carcinogens and the western diet and life style. Carcinogenesis is a multistage disease process that has been classified into initiation, promotion and progression stages; and each stage probably involves both genetic and epigenetic changes[66]. These observations have been substantiated experimentally by external administration of carcinogens[67]. Metabolic activation of carcinogen is a free-radical-dependent reaction. DNA damage mediated by free radicals plays a critical role in carcinogenesis[68,69]. In biological systems, damaged DNA is repaired enzymatically and cells regain their normal functions. However, misrepair of DNA damage may result in mutations such as base substitution and deletion, leading to carcinogenesis[70]. Sequence specificity of DNA damage plays a key role in the mutagenic process. Endogenous DNA damage arises from a variety of intermediates of oxygen reduction and several free radicals have been reviewed to take part in this process

by various mechanisms[71]. These reactive species have different redox potentials and redox potentials of these free-radical species may play an important role in sequence-specific DNA damage[17].

Apart from redox-potential of free-radical species, oxidation potential of DNA bases also contributes to the determination of sequence specificity of DNA damage. Guanine is most easily oxidized among the four DNA bases, as its oxidation potential is lowest (1.29 V vs normal hydrogen electrode) among others (adenine 1.42 V, cytosine 1.6 V and thiamin 1.7 V)[72,73]. Though the most common hydroxyl radical causes DNA damage with no marked site specificity, Kawanishi *et al.*[71] have delineated the mechanism of guanine-specific DNA damage by different free-radical entities and their role in carcinogenesis. Apart from a variety of free radicals, non-radical oxidant like H_2O_2 also play an important role in DNA damage. In biological systems, H_2O_2 is generated through spontaneous and/or superoxide dismutase (SOD) catalysed dismutation of $O_2^{\bullet}$. The $O_2^{\bullet}$ is produced by one electron reduction of molecular oxygen through reaction with free radicals and enzymatic reaction catalysed by xanthine oxidase. H_2O_2 has emerged as a pivotal molecule not only for cancer cell proliferation, but also in determining the fate of cancer cells exposed to phenolic phytochemicals. Higher amounts of ROS and H_2O_2 are produced in some cancer cells. The cumulative production of free radicals and H_2O_2 in human melanoma, neuroblastoma, colon carcinoma and ovarian carcinoma cell lines are comparable to that in phorbol ester-stimulated human blood neutrophils[74]. Since cancer cells constitutively produce high amounts of H_2O_2, the concept of persistent oxidative stress in cancer originated[75], which provides plausible explanation for some of the abnormal characteristics of cancer cells[76].

Recently, Loo[76] has reviewed redox-sensitive mechanisms of phytochemicals (particularly antioxidant polyphenols) mediated inhibition of cancer cell proliferation. Cancer cells, particularly those that are highly invasive or metastatic, require a certain level of oxidative stress to maintain a balance between undergoing either proliferation or apoptosis. They constitutively generate large but tolerable amounts of H_2O_2 that apparently function as signalling molecules in mitogen-activated protein kinase (MAPK) pathway to constantly activate redox-sensitive transcription factors and responsive genes that are involved in survival of cancer cells as well as their proliferation. With such a reliance of cancer cells on H_2O_2, it follows that if the excess H_2O_2 can be scavenged by phenolic phytochemicals having antioxidant activity, the oxidative stress-responsive genes can be suppressed and consequently proliferation of cancer cells can be inhibited. On the other hand, phenolic and other phytochemicals known as isothiocyanates, can induce the formation of H_2O_2 to achieve an intolerable level of high oxidative stress in cancer cells. As an early response, stress genes are activated; however, when critical threshold for cancer cells to cope with the induced oxidative stress reaches beyond tolerable limits, the key cellular components such as DNA suffer irreparable damage. In conjunction, genes involved in initiating cell-cycle arrest and/or apoptosis get activated. Therefore, the antioxidant phytochemicals can either scavenge constitutive H_2O_2 or paradoxically generate additional amounts of H_2O_2 to inhibit proliferation of cancer cells[76] and act as anticancer agents.

Apart from these actions, antioxidants have also been advocated to impart anticancer activities by several other mechanisms[77]: (i) Trapping the ultimate carcinogen, (ii) blocking the metabolic activation of carcinogens, (iii) modulating xenobiotic metabolizing enzymes, (iv) scavenging free radicals, (v) inhibiting generation of free radicals, (vi) inhibiting promotion stage of carcinogenesis by inhibiting cell proliferation through blocking lipoxygenase/cycloxygenase pathway or by lowering ornithine decarboxylase activity, and (vii) by decreasing the bioavailability of ultimate carcinogen, etc.

Insights towards developing novel antioxidants

The above discussions throw light on the involvement of some common pathways in pathogenesis of different diseases mediated through oxidative stress and free radicals (Figure 2). It has been observed there is some commonality in antioxidant molecules, i.e. presence of phenolic pharmacophore[3,23] (Figure 3).

Indeed, various kind of radicals like hydroxyl, alkoxyl, peroxyl and carbon centred, have been observed to be involved in oxidative stress *in vivo*, Niki *et al.*[78] suggest that considering the activities of radicals and concentration of substrates, the peroxyl radicals should be the major target radical for radical scavenging antioxidants *in vivo*. Activity of phenolic antioxidants towards peroxyl radicals is determined primarily by BDE of phenolic O–H bond, its redox potential and steric hindrance to abstraction of phenolic hydrogen by peroxyl radicals[79,80]. Peroxyl radicals are electrophilic in nature. Therefore, electron-donating substituents on the aromatic ring of the phenolic pharmacophore increases the reactivity towards peroxyl radical, whereas electron-withdrawing groups decreases it. In addition to such polar effects, the electron-donating substituents also weaken phenolic O–H bond, while electron-withdrawing substituents increases O–H bond dissociation energies. Therefore, it is important that an antioxidant should have electron-donating substituents[77]. Furthermore, substituents on the ortho-position are important in determining the stability of phenoxyl radical, hindrance for approach of peroxyl radicals to the phenolic hydrogen, and the reactivity of phenoxyl radical towards the substrate[81]. Flavanols, present in abundance in dietary constituents, contain a strong nucleophilic centre that reacts with electrophilic species and thereby decreases the

bioavailability of the ultimate carcinogens[82]. Therefore, presence of nucleophilic electron-donating properties of polyphenols present in tea may be one of the important mechanisms of action involved in inhibition of carcinogenesis, where electrophilic carcinogenic species may be trapped by nucleophilic polyphenols[77].

Considering these criteria and antioxidant property as the base in view, several novel molecules have emerged for development as therapeutics in various multifactorial diseases like atherosclerosis[23,28,29,83,84], stroke[37], cancer[17,76], and simultaneously, several insights have been provided for therapeutic advancement for AD and PD[51,52].

Diphenyl picrylhydrazyl (DPPH) is a nitrogen-centred free radical. It reacts similar to the peroxyl radical. Its reaction rates correlate directly with antioxidant activity. Higher the rate, more effective the antioxidant[17]. Two mechanisms for antioxidants to scavenge DPPH radical have been proposed[85]: the first is a direct H-atom abstraction process (eq. (1)) and the second is a proton concerted electron-transfer process (eq. (2))

$$DPPH^{\bullet} + RXH \rightarrow DPPHH + RX^{\bullet}, \quad (1)$$

$$DPPH^{\bullet} + RXH \rightarrow DPPH^{-} + RXH^{\bullet+} \rightarrow DPPHH + RX^{\bullet}. \quad (2)$$

In which, X represents either O, N, S or C. First pathway is governed to a large extent by X–H BDEs, of RXH and DPPHH. Only if the BDE of former is lower than that of the latter, the reaction is permitted. The BDE for DPPHH is calculated to be 172.22 kcal/mol. While, the second pathway is determined by ionization potentials (IP) of RXH and $DPPH^{-}$. The prerequisite for this reaction to proceed is that IP of the RXH should be lower than that of $DPPH^{-}$. The IP for $DPPH^{-}$is observed to be 59.60 kcal/mol. Phenol, amino, or thiophenol groups are commonly known to be the active groups for scavenging DPPH. These understandings may provide better insights in designing the active centers for antioxidants as radical scavenger and by protecting these centers, further modify structures to improve the absorption and metabolic properties in the molecule.

Edaravone is a novel neuroprotective agent approved for acute therapy of embolic stroke. The pharmacological effect of it arises from its radical scavenging activity. However, it does not possess phenolic pharmacophore. It has been observed recently that edaravone scavenges DPPH through donating H-atom[85], as the C–H BDEs of edaravone (77.26–86.36 kcal/mol) are much lower than DPPHH. However, it cannot scavenge DPPH by second method as its IP (164.72 kcal/mol) is much higher than $DPPH^{-}$.

Antioxidants tested on DPPH were also found extremely effective in cell systems of oxidative stress used to test anticancer agents[17]. This simple test further provides information on the ability of a compound to donate electrons, the number of electrons a given molecule can donate and on the mechanism of antioxidant action. Furthermore, in cases where the structure of the electron-donor is not known (e.g. a plant extract), this method can afford data on the reduction potential of the sample, and hence can be helpful in comparing the reduction potential of unknown materials. Vaya *et al.*[86] observed that compounds which were able to donate electrons to the DPPH molecule were the same as those that showed high activity in inhibiting LDL oxidation induced under different conditions. Similarly, among several mechanisms for the development of diabetic complications, increased level of advanced glycation end-products (AGEs) are well known to be a cause of aging and diabetic complications. Matsuda *et al.*[87] and Lou *et al.*[88] have observed that the AGEs formation inhibitory activities of several flavonoids were in accordance with their DPPH radical scavenging activities. Therefore, this simple test model may be helpful in identifying antioxidant resources as well as molecules useful for development of anticancer, antiatherosclerotic, antidiabetic therapeutics and neuroprotective agents. Applying this model as prerequisite, it has been possible for us to identify several traditional medicinal preparations and medicinal plants bearing rich content of antioxidants[89], isolate a number of molecules[90–93]. Furthermore, we also improved and incorporated several biological activities in that molecules[92]. These medicinal plants have been used for a variety of disease conditions now being explained by oxidative stress theory. These resources/medicinal plants therefore, may provide an important base and molecules as building blocks for development of indigenous therapies for disease of polygenic origin.

Future scenario

Technology-based economic growth has been one of the prime factors in creating the wealth of a nation. The most developed countries are characterized by their wealth creation based on pursuing high quality research and development investments and translating their innovations into commercial products[94]. These criteria appear tough for the developing nations, as they are poorly prepared to invest large sums of money for advanced research and development. Therefore, developing countries like India may find a solution by looking back into their glorious past of traditional medicinal practice like Ayurveda, Unani and Siddha for alternative therapeutic options.

Ayurveda and Siddha, discovered, nurtured and perfected in India as science of longevity, are not just a collection of therapeutic recipes, but also frameworks that define the condition of sickness and connect them with healing practices. In olden days these scientific disciplines not only thrived in India but also influenced healing practices in many other countries. That period of intense creativity was a glorious one and every Indian has the

reason to remember it with pride[95]. However, after January 2005, when new intellectual property regulations come into force, Indian companies may no longer copy drugs, but will have to develop them on their own. As an alternative therefore, they may rely on the traditional medical knowledge and biodiversity as springboards. By fusing ancient wisdom and modern science, India can create world-class products[96]. 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 resources of the country[89]. 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[96]. Identification of antioxidant-rich natural resources, preparing molecular fingerprints of their chemical compositions and studying the multiple therapeutic properties in this programme may help make India self-reliant in drug development in future.

1. Lehninger, A. L., Nelson, D. L. and Cox, M. M., *Principle of Biochemistry*, Worth Publishers, New York, 1990, 2nd edn, p. 359.
2. Tiwari, A. K., Imbalance in antioxidant defence and human disease: multiple approach of natural antioxidants therapy. *Curr. Sci.*, 2001, **81**, 1179–1187.
3. Noguchi, N., Watanabe, A. and Shi, H., Diverse functions of antioxidants. *Free Radic. Res.*, 2000, **33**, 809–817.
4. Sies, H., Oxidative stress; introductory remarks. In *Oxidative Stress* (ed. Sies, H.), Academic Press, London, 1982, pp. 1–8.
5. Finkel, T., Oxidants, oxidative stress and the biology of aging. *Nature*, 2000, **408**, 239–248.
6. Davies, K. J. A., Oxidative stress, antioxidant defenses and damage removal, repair, and replacement system. *IUBMB Life*, 2000, **50**, 279–289.
7. Nose, K., Role of reactive oxygen species in the regulation of physiological functions. *Biol. Pharm. Bull.*, 2000, **23**, 897–903.
8. Papas, A. M., Determinants of antioxidants status in humans. *Lipids*, 1996, **31**, S77–S82.
9. Polidori, M. C. *et al.*, Profiles of antioxidants in human plasma. *Free Radic. Biol. Med.*, 2001, **30**, 456–462.
10. Tiwari, A. K., Natural product antioxidants and their therapeutic potential in mitigating peroxidative modification of lipoproteins and atherosclerosis: recent developments. *J. Med. Aromat. Plant Sci.*, 1999, **21**, 730–741.
11. Halliwell, B., Free radicals, antioxidants and human diseases: curiosity, cause and consequences. *Lancet*, 1994, **344**, 721–724.
12. Bruckdorfer, R. K., Antioxidants, lipoprotein oxidation, and arterial function. *Lipids*, 1996, **31**, S83–S85.
13. Pietta, P. G., Flavonoids as antioxidants. *J. Nat. Prod.*, 2000, **63**, 1035–1042.
14. Chipault, J. R., Antioxidants for use in food. In *Autooxidation and Antioxidants* (ed. Lundberg, W. O.), Interscience, New York, 1962, vol. II, pp. 477–542.
15. Halliwell, B., How to characterize biological antioxidants. *Free Radic. Res. Commun.*, 1990, **9**, 1–32.
16. Frankel, E. N. and Meyer, A. S., The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.*, 2000, **80**, 1925–1941.
17. Wright, J. S., Searching for fountain of youth. *Chem. Br.*, 2003, **39**, 25–27.
18. Fang, Y. Z., Yang, S. and Wu, G., Free radicals, antioxidants and nutrition. *Nutrition*, 2002, **18**, 872–879.
19. Morganti, M. *et al.*, Atherosclerosis and cancer: common pathways on vascular endothelium. *Biomed. Pharmacother.*, 2002, **56**, 317–324.
20. Brown, M. S. and Goldstein, J. L., Lipoprotein metabolism in the macrophage: implication of cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.*, 1983, **52**, 223–261.
21. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. and Witztum, J. L., Beyond cholesterol: modification of low density lipoprotein that increases its atherogenesity. *N. Engl. J. Med.*, 1989, **320**, 915–924.
22. Tiwari, A. K., Pathogenesis of atherosclerosis: peroxidation of lipoprotein lipids and use of antioxidants; current developments. *Ann. Natl. Acad. Med. Sci. (India)*, 1996, **32**, 87–95.
23. Noguchi, N., Novel insights into the molecular mechanisms of the antiatherosclerotic properties of antioxidants: The alternative to radical scavenging. *Free Radic. Biol. Med.*, 2002, **33**, 1480–1489.
24. Sreekumar, R. *et al.*, Impact of high fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle. *Am. J. Physiol. Endocrinol. Metab.*, 2002, **282**, E1055–E1061.
25. Walldius, G. *et al.*, The effect of Probucol on femoral atherosclerosis: The Probucol Quantitative Regression Swedish Trial. *Am. J. Cardiol.*, 1994, **74**, 875–883.
26. Duell, P. B., Prevention of atherosclerosis with dietary antioxidants. Facts or fiction. *J. Nutr.*, 1996, **126**, 1067S–1071S.
27. Steinberg, D., Clinical trials of antioxidants in atherosclerosis: Are we doing the right thing? *Lancet*, 1995, **346**, 36–38.
28. Cynshi, O. *et al.*, Antiatherogenic effects of antioxidant BO-653 in three different animal models. *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 10123–10128.
29. Wasserman, M. A. *et al.*, Chemistry and biology of vascular protactants: a novel approach to the treatment of atherosclerosis and coronary artery disease. *Am. J. Cardiol. (Suppl.)*, 2003, **91**, 34A–40A.
30. Tiwari, A. K., Singh, P. N., Mishra, B. and Gupta, R. P., Cardioprotective properties of *Terminalia arjuna*: evaluation of its antihyperlipidemic and antiatherosclerotic properties. In *Chemistry and Biology of Herbal Medicine* (eds Kamboj, V. P. and Agrawal, V. P.), Society of Bioscience Publication, Muzzafarnagar, 1997, pp. 193–198.
31. Tiwari, A. K., Gode, J. D. and Dubey, G. P., Effect of *Terminalia arjuna* on lipid profiles of rabbits fed hypercholesterolemic diet. *Int. J. Crude Drug Res.*, 1990, **28**, 43–47.
32. Tiwari, A. K., Gode, J. D. and Dubey, G. P., Influence of Abana on experimental atherogenesis in hypercholesterolemic rabbits. *Jpn. Heart J.*, 1993, **34**, 451–458.
33. Tiwari, A. K., Shukla, S. S., Agrawal, A. and Dubey, G. P., Lowering serum cholesterol to high density lipoprotein cholesterol ratio in hypercholesterolemic patients by Abana: possible cardioprotective action. *Alternative Med.*, 1990, **3**, 145–148.
34. Inoue, M., Shosaikoto as a potential antiatherosclerotic agent. *Drug News Perspect.*, 2000, **13**, 407–412.
35. Sundaram, V., Hanna, A. N., Lubow, K. L., Falko, J. M. and Sharma, H. M., Inhibition of low density lipoprotein oxidation by oral herbal mixtures MAK-4 and MAK-5 in hyperlipidemic patient. *Am. J. Med. Sci.*, 1997, **314**, 303–310.
36. Libby, P., Atherosclerosis: The new view. *Sci. Am.*, 2002, **286**, 28–37.
37. Sorbera, L .A., Leeson, P. A., Castaner, J. and del Frenso, M., NXY-059. Treatment of ischaemic stroke, Free radical scavenger. *Drugs Future*, 2002, **27**, 240–247.
38. Evans, J. L., Goldfine, I. D., Maddux, B. A. and Grodsky, G. M., Oxidative stress and stress activated signaling pathways: a unifying hypothesis of type-2 diabetes. *Endocr. Rev.*, 2002, **23**, 599–622.

39. Strowing, S. M. and Raskin, P., Glycemic control and the complications of diabetes: after the diabetes control and complication trial. *Diabetes Rev.*, 1995, **3**, 237–257.
40. Baynes, J. W. and Thrope, S. R., The role of oxidative tress in diabetic complications. *Curr. Opin. Endocrinol.*, 1996, **3**, 277–284.
41. Karasu, C., Ozansoy, G., Bozkurt, O., Erdogan, D. and Omeroglu, S., Antioxidant and triglyceride lowering effect of vitamin E associate with the prevention of abnormalities in the reactivity and morphology of aorta from streptozotocin-diabetic rats. *Metabolism*, 1997, **46**, 872–879.
42. Kocak, G. *et al.,* Alpha lipoic acid treatement ameliorats metabolic parametres, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes (The ADIC Study group). *Diabetes Nutr. Metab.*, 2000, **13**, 308–319.
43. Jain, S. K., McVie, R. and Smith, T., Vitamin E supplementation restores glutathione and malonaldehyde to normal concentrations in erythrocytes of type-1 diabetic children. *Diabetes Care*, 2000, **23**, 1389–1394.
44. Pekiner, B., *et al.*, *In vivo* treatement with stobadine prevents lipid peroxidation, protein glycation and calcium overload but does not ameliorate Ca^{++}-ATPase activity in heart and liver of streptozotocin-diabetic rats: comparison with vitamin E. *Biochim. Biophys. Acta*, 2002, **1588**, 71–78.
45. Kowluru, R. A., Engerman, R. L. and Kern, T. S., Diabetes induced metabolic abnormalities in myocardium: effect of antioxidant therapy. *Free Radic. Res.*, 2000, **32**, 67–74.
46. Jain, S. K. and Lim, G., Pyridoxine and pyridoxamine inhibits superoxide radicals and prevents lipid peroxidation, protein glycation, and $(Na^{+}+K^{+})$-ATPase activity reduction in high glucose treated human erythrocytes. *Free Radic. Biol. Med.*, 2001, **30**, 232–237.
47. Horakova, L. and Stolc, S., Antioxidant and pharmacodynamics effects of pyridoindole ST. *Gen. Pharmacol.*, 1998, **30**, 627–638.
48. Tiwari, A. K. and Rao, J. M., Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. *Curr. Sci.*, 2002, **83**, 30–38.
49. Scartezzini, P. and Speroni, E., Review of some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol.*, 2000, **71**, 23–43.
50. Mc Cune, L. M. and Johns, T., Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous people of the North American boreal forest. *J. Ethnopharmacol.*, 2002, **82**, 197–205.
51. Esposito, E., Ratilio, D., Di Matteo, V., Di Glulia, C., Cacchio, M. and Algeri, S., A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiol. Aging*, 2002, **23**, 719–735.
52. Behl, C., Alzheimer's disease and oxidative stress: implications for novel therapeutic approach. *Prog. Neurobiol.*, 1999, **57**, 301–323.
53. Foley, D. J. and White, L. R., Dietary intake of antioxidants and risk of Alzheimer disease: food for thought. *JAMA*, 2003, **287**, 3261–3263.
54. Rocca, W. A. *et al.*, Frequency and distribution of Alzheimer's disease in Europe; a collaborative study of 1980–1990 prevalence findings. *Ann. Neurol.*, 1991, **30**, 381–390.
55. Hofman, A. *et al.*, The prevalence of dimentia in Europe: a collaborative study of 1980–1990 findings. *Int. J. Epidemiol.*, 1991, **20**, 736–748.
56. Harman, D., Eddy, D. E. and Naffsinger, J., Free radical theory of aging: inhibition of amyloidosis in mice by antioxidants; possible mechanism. *J. Am. Geriatr. Soc.*, 1976, **24**, 203–210.
57. Engelhart, M. J. *et al.*, Dietary intake of antioxidants and risk of Alzheimer's disease. *JAMA*, 2003, **287**, 3223–3229.
58. Sano, M. *et al.*, A controlled trial of selegiline, alpha tocopherol, or both as treatment for Alzheimer's disease. *N. Engl. J. Med.*, 1997, **336**, 1216–1222.
59. Scherman, D., Desnos, C., Darchen, F., Pollak, P., Javoy-Agid, F. and Agid, Y., Striatal dopamine deficiency in Parkinson's disease; role of aging. *Ann. Neurol.*, 1989, **26**, 551–557.
60. Olanow, C. W. and Tatton, W. G., Etiology and pathogenesis of Parkinson's disease. *Annu. Rev. Neurosci.*, 1999, **22**, 123–144.
61. Jenner, P., Dexter, D. T., Sian, J., Schapira, A. H. V. and Marsolen, C. D., Oxidative stress as a cause of nigral cell death in Parkinson's disease and incident Lewy body disease. *Ann. Neurol.*, 1992, **32**, 582–587.
62. Gao, H. M., Liu, B., Zhang, W. and Hong, J. S., Novel antiinflammatory therapy for Parkinson' disease. *Trends in Pharmacol. Sci.*, 2003, **24**, 395–401.
63. Parkinson Study Group, Mortality in DATATOP: a multicenter trial in early Parkinson's disease. *Ann. Neurol.*, 1998, **43**, 318–325.
64. Fahn, S., An open trial of high dosage antioxidants in early Parkinson's disease. *Am. J. Clin. Nutr.*, 1991, **53**, 380S–382S.
65. Eaton, L., World cancer rates set to double by 2020. *Br. Med. J.*, 2003, **326**, 728.
66. Bishop, J. M., Molecular themes in oncogenesis. *Cell*, 1991, **64**, 235–248.
67. Wattenberg, L. W., What are the clinical attributes for cancer chemotherapy. *Ann. N.Y. Acad. Sci.*, 1995, **768**, 73–81.
68. Guyton, K. Z. and Kensler, T. W., Oxidative mechanisms in carcinogenesis. *Br. Med. Bull.*, 1993, **49**, 523–544.
69. Feig, D. I., Reid, T. M. and Loeb, L. A., Reactive oxygen species in tumorigenesis. *Cancer Res. (Suppl. 7)*, 1994, **54**, 1890–1894.
70. Poulsen, H. E., Prieme, H. and Loft, S., Role of oxidative DNA damage in cancer initiation and promotion. *Eur. J. Cancer Prev.*, 1998, **7**, 9–16.
71. Kawanishi, S., Hiraku, Y. and Oikawa, S., Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutat. Res.*, 2001, **488**, 65–76.
72. Burrows, C. J. and Muller, J. G., Oxidative nucleobase modifications leading to strand scission. *Chem. Rev.*, 1998, **98**, 1109–1151.
73. Steenken, S. and Jovanovic, S., How easily oxidizable is DNA? One electron reduction potential of adenosine and guanosine radicals in aqueous solution. *J. Am. Chem. Soc.*, 1997, **119**, 617–618.
74. Szatrowski, T. P. and Nathan, C. F., Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res.*, 1991, **51**, 794–798.
75. Toyokuni, S., Okamoto, K., Yodoi, J. and Hiai, H., Persistent oxidative stress in cancer. *FEBS Lett.*, 1995, **358**, 1–3.
76. Loo, G., Redox sensitive mechanisms of photochemical-mediated inhibition of cancer cell proliferation. *J. Nutr. Biochem.*, 2003, **14**, 64–73.
77. Smith, T. J., Hong, J. Y., Wong, Z. Y. and Yang, C. S., How can carcinogenesis be inhibited. *Ann. N.Y. Acad. Sci.*, 1995, **768**, 82–90.
78. Niki, E., Noguchi, N., Tsuchihashi, H. and Gotoh, N., Interaction among vitamins C, vitamin E, and β-carotene. *Am. J. Clin. Nutr.*, 1995, **62**,1322S–1326S.
79. Burton, G. W. *et al.*, Autoxidation of biological molecules, 4. Maximizing the antioxidant activity of phenols. *J. Am. Chem. Soc.*, 1985, **107**, 7053–7065.
80. Mukai, K., Okabe, K. and Hosose, H., Synthesis and stop flow investigation of antioxidant activity of tocopherol. Finding of new tocopherol derivatives having highest antioxidant activity among phenolic antioxidants. *J. Org. Chem.*, 1989, **54**, 557–560.
81. Noguchi, N. and Niki, E., Phenolic antioxidants: A rational for design and evaluation of novel antioxidant drug for atherosclerosis. *Free Radic. Biol. Med.*, 2000, **28**, 1538–1546.
82. Yang, C. S. and Wang, Z. Y., Tea and Cancer. *J. Natl. Cancer Inst.*, 1993, **85**, 1038–1048.
83. Batra, S., Srivastava, S., Singh, K., Chander, R., Khanna, A. K. and Bhaduri, A. P., Synthesis and biological evaluation of 3-substitued amino-1-aryl-6-hydroxy-hex-2-ene-1-ones as antioxidant and hypolipidemic agents. *Bioorg. Med. Chem.*, 2000, **8**, 2195–2209.

84. Lee, S. *et al.*, Antiatherogenic effects of 3,4-dihydroxyhydrocinnamides. *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2681–2682.
85. Wang, L. F. and Zhang, H. Y., A theoretical investigation on DPPH radical-scavenging mechanism of Edaravone. *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3789–3792.
86. Vaya, J. *et al.*, Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. *Phytochemistry*, 2003, **62**, 89–99.
87. Matsuda, H., Wang, T., Managi, H. and Yoshikawa, M., Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg. Med. Chem.*, 2003, **11**, 5317–5323.
88. Lou, H., Yuan, H., Yamazaki, Y., Sasaki, T. and Oka, S., Alkaloids and flavonoids from peanut skin. *Planta Med.*, 2001, **67**, 345–349.
89. CSIR coordinated program on 'Development and commercialization of bioactive substances from plant sources', New Delhi, India.
90. Tiwari, A. K., Srinivas, P. V., Kumar, S. P. and Rao, J. M., Free radical scavenging active components from *Cedrus deodara*. *J. Agric. Food Chem.*, 2001, **49**, 4642–4645.
91. Sampath Kumar, U., Tiwari, A. K., Reddy, S. V., Rao, R. J., Ali, Z. and Rao, J. M., Bioassay guided isolation of free radical scavenging and xanthine oxidase inhibitory compounds from *Stereospermum personatum*. In *Utilization of Bioresources* (eds Sree, A., Rao, Y. R., Nanda, B. and Mishra, V. N.), Allied Publishers Pvt. Ltd., New Delhi, 2002, pp. 32–37.
92. Rao, R. J., Tiwari, A. K., Sampath Kumar, U., Reddy, S. V., Ali, Z. A. and Rao, J. M., Novel 3-O-acyl mesquitol analogues as free radical scavengers and enzyme inhibitors: synthesis, biological evaluation and structure activity relationship. *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2777–2780.
93. Reddy, S. V., Tiwari, A. K., Kumar, S. P., Rao, R. J. and Rao, J. M., Free radical scavenging, enzyme inhibitory constituents from antidiabetic traditional Indian medicinal plant *Hydnocarpus wightiana* Blume. *Phytother. Res.*, 2004, in press.
94. Bhattacharya, S. and Nath, P., Using patent statistics as a measure of 'technological assertiveness': A China India comparison. *Curr. Sci.*, 2002, **83**, 23–29.
95. Kumar, R., How to globalize Ayurveda, Siddha and Unani. *Chem. Ind. News*, 2000, **XLV**, 561–563.
96. Jayaraman, K. S., Technology, tradition unite India's drug discovery scheme. *Nature Med.*, 2003, **9**, 982.

ACKNOWLEDGEMENTS. This work is supported by the coordinated programme on 'Development and commercialization of bioactive substances from plant sources', of the CSIR, New Delhi. I thank Dr J. S. Yadav, Director, IICT for his keen interest, and Dr. J. M. Rao, Organic-I Division, and Dr P. V. Diwan, Pharmacology Division, IICT for constructive suggestions and inputs. Secretarial assistance from Mr S. A. Hafeez, technical assistance from Mrs A. Z. Ali is acknowledged. IICT communication No 040112.

Received 12 November 2003; accepted 14 January 2004