review

structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure

Swaran J.S. Flora

Defense Research and Development Establishment; Division of Pharmacology and Toxicology; Gwalior, India

Abstract

Oxidative stress contributes to the pathophysiology of exposure to heavy metals/metalloid. Beneficial renal effects of some medications, such as chelation therapy depend at least partially on the ability to alleviate oxidative stress. The administration of various natural or synthetic antioxidants has been shown to be of benefit in the prevention and attenuation of metal induced biochemical alterations. These include vitamins, N-acetylcysteine, α-lipoic acid, metronidazol, dietary flavonoids and many others. Human studies are limited in this regard. Under certain conditions, surprisingly, the antioxidant supplements may exhibit pro-oxidant properties and even worsen metal induced toxic damage. To date, the evidence is insufficient to recommend antioxidant supplements in subject with exposure to metals. Prospective, controlled clinical trials on safety and effectiveness of different therapeutic antioxidant strategies either individually or in combination with chelating agent are indispensable. The present review focuses on structural, chemical and biological aspects of antioxidants particularly related to their chelating properties.

Introduction

Oxidation is a chemical reaction involving transfer of an electron from electron rich to electron deficient entity. The electron deficient molecule is termed an oxidizer or oxidizing agent. Heavy metals due to the presence of vacant d-orbital behave as potent oxidizing agents. Metals and metal compounds are natural constituents of all ecosystems, moving between biological and non-biological system. Numerous studies have reported toxic and carcinogenic effects induced when humans and animals are exposed to certain metals especially arsenic, lead, cadmium, chromium and mercury. A growing amount of results provide evidence that toxic and carcinogenic metals are capable of interacting with nuclear proteins and deoxyribonucleic acid (DNA) causing oxidative deterioration of biological macromolecules. Detailed studies in the past two decades have shown that metals like iron, cadmium, chromium, mercury, arsenic and lead possess the ability to produce reactive free radical species, which start chain reaction resulting in oxidation of lipid termed as lipid peroxidation, protein oxidation and oxidation of nucleic acid like DNA and ribonucleic acid (RNA). Deleterious free radical-mediated oxidations occur in aerobic organism as a result of normal oxygen metabolism.

An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Generally, an antioxidant can protect against metal toxicity by trapping free radicals thus terminating the chain reaction, by chelating metal ion and preventing the reaction with reactive oxygen species or by chelating metal and maintaining it in a redox state leading to its incompetency to reduce molecular oxygen. Substances which protect biomolecules from free radical-mediated damage both in vivo and in vitro fall under this category. Reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS): (1) are generated during irradiation by UV light, by X-rays and by gamma rays; (2) are products of metal-catalyzed reactions; (3) are present as pollutants in the atmosphere; (4) are produced by neutrophils and macrophages during inflammation; (5) are by-products of mitochondria-catalyzed electron transport reactions and other mechanisms. ROS at higher concentration are important mediators of damage to cell cell structures, including lipids and membranes, proteins and nucleic acids (termed oxidative stress). The harmful effects of ROS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes.

Key words: chelation therapy, oxidative stress, antioxidant, metal toxicity, combination therapy

Abbreviations:

DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ROS, reactive oxygen species; RNS, reactive nitrogen species; NO, nitric oxide; ALAD, δ-aminolevulinic acid dehydratase; TBARS, thiobarbituric acid reactive substance; oxyHb, oxyhaemoglobin; GSH, glutathione; EDTA, ethylenediamine tetra acetic acid; DMSA, meso 2,3-dimercaptosuccinic acid; MiADMSA, monoisoamyl dimercaptosuccinic acid; NOAEL, no observed adverse effect level; LA, α-lipoic acid; DHLA, dihydrolipoic acid; PUFA, poly unsaturated fatty acids; AA, ascorbic acid; DHAA, dihydroascorbic acid; NAC, N-acetyl-L-cysteine

Correspondence to: Swaran J.S. Flora; Division of Pharmacology and Toxicology; Defence Research and Development Establishment; Jhansi Road, Gwalior 474 002 India; Email: sjflora@hotmail.com

Submitted: 05/23/09; Revised: 05/28/09; Accepted: 05/28/09

Previously published online as an Oxidative Medicine and Cellular Longevity E-publication:
http://www.landesbioscience.com/journals/oximed/article/9112

Oxidative Medicine and Cellular Longevity 2009; 2:4; 191-206; September/October 2009; ©2009 Landes Bioscience

www.landesbioscience.com Oxidative Medicine and Cellular Longevity 191
This paper examines a discussion of the various protective pathways that may be provided by the antioxidant network against the deleterious action of free radicals.

**Chemistry and Biochemistry of Free Radicals**

**Reactions of free radicals.** Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons.\(^\text{11}\) Although this definition does not specify exactly where the unpaired electron is present, still it is preferred because it allows us to classify most of the transition metal ions as free radicals and thus better understand the close interrelation between oxygen and reactive metal ions. Due to presence of this unpaired electron, these radicals confer a considerable degree of reactivity.

Figure 1 shows the generation of some of the reactive oxygen species. The hydroperoxyl radical formed above dissociates to give superoxide anion radical at physiological pH 7.4. Superoxide anion can further interact with other molecules to generate other ROS either directly or prevalently through enzyme- or metal-catalyzed processes (\(\text{O}_2^-\)). Superoxide ion is rapidly detoxified initially to hydrogen peroxide because of its dismutation reaction to form hydrogen peroxide and oxygen\(^\text{12}\) and finally to water by Cu, Zn-SOD and/or Mn-SOD.

![Figure 1. Redox reaction showing generation of various Reactive Oxygen Species (ROS).](image)

Significant amounts of hydrogen peroxide are formed in the mitochondria and this is enriched with antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPxs) which minimizes the oxidative stress.\(^\text{13}\)

\[
2\text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]

Fe (II) can participate in the Fenton reaction, generating highly reactive hydroxyl radical. Thus under stress conditions, \(\text{O}_2^-\) facilitates OH\(^+\) production by \(\text{H}_2\text{O}_2\) by making Fe (II) available for the Fenton reaction. On the other hand the superoxide radical participates in the Haber-Weiss reaction which combines a Fenton reaction and the reduction of Fe (III) by superoxide, yielding Fe (II) and oxygen.

![Figure 2. Effects of ROS on DNA damage leading to its role in carcinogenesis.](image)

**Metal Induced Oxidative Stress**

Numerous studies have focused on metal-induced toxicity and carcinogenicity, emphasizing their role in the generation of reactive oxygen and nitrogen species in biological systems, and the significance of this therein. Metal-mediated formation of free radicals may cause various modifications to DNA bases as well as can enhance lipid peroxidation (Fig. 2).

Oxidative stress, a condition describing the production of oxygen radicals beyond a threshold for proper antioxidant neutralization, has been implicated as a pathologic condition in several cellular disorders. Besides ROS, metal exposure can also affect the generation of RNS. Nitric oxide (NO) is a messenger molecule that plays an important role in neurotransmission, vasodilation and immune response.\(^\text{14}\) NO also possesses toxic effects such as pro-oxidant effects, genotoxicity and mutagenicity. Production of NO is catalyzed mainly by NO synthases, which consist of neuronal, endothelial and inducible forms.\(^\text{15}\)

A number of metals have been long known in literature for their toxicity and carcinogenicity. Increased amounts of iron in the body poses enhanced risk of a variety of diseases including vascular disease, cancer and certain neurological conditions.\(^\text{16,17}\) Copper at high concentration is known to cause metastasis of cancer cells.\(^\text{18}\) Cobalt(II) complexes are known to produce oxygen radicals which causes heart toxicity.\(^\text{19,20}\)

Arsenic is one of the most toxic elements and produces a variety of ROS including superoxide (\(\text{O}_2^-\)), singlet oxygen (\(\text{(^1}\text{O}_2\)), the peroxyl radical (\(\text{ROO}^-\)), nitric oxide (\(\text{NO}^-\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), dimethylarsenic peroxyl radicals ([\(\text{CH}_3\text{AsOO}^-\)]) and also the dimethylarsenic radical ([\(\text{CH}_3\text{As}^-\)]).\(^\text{21-24}\) As (III) enhances the production of heme oxygenase, an indicator of oxidative stress in a variety of human and mammalian cell types\(^\text{25}\) and generates free radicals in livers of mice.\(^\text{26}\) The production of ROS by various arsenic metabolites was confirmed by animal experiments.\(^\text{27}\)

Different mechanisms have been accounted for the toxicity of arsenic. Arsenic (III) compounds bind to sulphydryl (-SH) groups and can inhibit various enzymes, including glutathione reductase while Arsenic (V) inhibits PDH\(^\text{28}\) activity thus causing impaired glucose metabolism.\(^\text{28,29}\)

Studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and weakening the antioxidant defence system of cells.\(^\text{30-32}\) Depletion of cell’s major sulphydryl reserves e.g., \(\delta\)-aminolevulinic acid dehydratase (ALAD) seems to be an important indirect mechanism for oxidative stress.
Antioxidants and metal toxicity

Table 1  Alphabetical classification of antioxidants, their categories with few examples

| Alphabetical name | Categories of antioxidants | Examples |
|-------------------|---------------------------|----------|
| Antioxidant C     | Carotenoids               | β-carotene, lycopene, lutein |
| Antioxidant E     | Enzymes                   | SOD, Catalase, GPx |
| Antioxidant G     | Glutathione               | Glutathione |
| Antioxidant H     | Hormones                  | Melatonin, Oestrogen |
| Antioxidant L     | Lipid associated chemicals| Ubiquinol-10, N-acetyl cysteine, lipoic acid |
| Antioxidant M     | Minerals                  | Zinc, Selenium, Copper |
| Antioxidant P     | Phenolics                 | Quercetin, Catechin |
| Antioxidant S     | Saponines, Steroids       | Cortisone, Estradiol, Estril |
| Antioxidant V     | Vitamins                  | α-tocopherol, Ascorbic acid |

Figure 3. Enzymatic and non enzymatic classification of antioxidants with few.

that is induced by redox-inactive metals. Further, zinc which usually serves as a cofactor of many enzymes could be replaced by lead, thereby making the enzyme inactive. The increased lipid peroxidation and inhibition of enzymes responsible to prevent such oxidative damage have demonstrated lead induced oxidative injury. Lead induced disruption of the pro-oxidant/antioxidant balance could induce injury via oxidative damage to critical biomolecules. A significant decrease in the activity of tissue superoxide dismutase (SOD), a free radical scavenger and metalloenzymes (zinc/copper) on lead exposure has been reported. Catalase is an efficient decomposer of H₂O₂ and known to be susceptible to lead toxicity. Lead induced decrease in brain GPx activity may arise as a consequence of impaired functional groups such as glutathione (GSH) and NADPH or selenium mediated detoxification of toxic metals. Antioxidant enzyme glutathione S-transferase (GST) is known to provide protection against oxidative stress and the inhibition of this enzyme on lead exposure might be due to the depletion in the status of tissue thiol moiety.

These enzymes are important for maintaining critical balance in the glutathione redox state. Malondialdehyde (MDA) levels were strongly correlated with lead concentration in the tissues of lead exposed rats. The concentration of thiobarbituric acid reactive substance (TBARS), which is a reflection of endogenous lipid oxidation level, gets increased on lead exposure. The interaction of lead with oxyhaemoglobin (oxyHb) has been suggested as an important source of superoxide radical formation in RBCs.

Also studies have demonstrated the reactions of hydroxyl radicals which leads to abstraction of a hydrogen atom from the protein polypeptide backbone to form a carbon-centered radical, which under aerobic conditions reacts readily with dioxygen to form peroxyl radical. Metal-catalyzed damage to proteins involves oxidative scission, loss of histidine residues, bityrrosine crosslink, the introduction of carbonyl groups, and the formation of protein-centered alkyl, R⁺, alkoxy, RO⁻ and alkyloxy, ROO⁻, radicals.

Nomenclature and Classification of Antioxidants

Table 1 lists categories in which various antioxidants have been characterized based on their structure, occurrence and mode of action, solubility and kinetics.

Kinetically antioxidants can be classified into six categories as below:

1. Antioxidants that break chains by reacting with peroxy radicals having weak O-H or N-H bonds: phenol, naphthol, hydroquinone, aromatic amines and aminophenols.
2. Antioxidants that break chains by reacting with alkyl radicals: quinones, nitrones, iminoquinones.
3. Hydro peroxide decomposing antioxidants: sulphide, phosphide, thiophosphate.
4. Metal deactivating antioxidants: diamines, hydroxyl acids and bifunctional compounds.
5. Cyclic chain termination by antioxidants: aromatic amines, nitroxy radical, variable valence metal compounds.
6. Synergism of action of several antioxidants: phenol sulphide in which phenolic group reacts with peroxy radical and sulphide group with hydro peroxide.

Antioxidants as chelating agents and their mechanism of action. Non-enzymatic antioxidants as well as antioxidant enzymes (Fig. 3) are known to counteract the effect of ROS and RNS. These antioxidants are known to diffuse free radicals leading to limited risk of oxidative stress. At cellular and molecular level they inactivate ROS and under specific low concentration inhibit or delay oxidative processes by interrupting the radical chain reaction. Antioxidants also chelate the metal ions responsible for the generation of ROS as they have the potentials to work in both aqueous and/or membrane domains.

Chelation is the formation of a metal ion complex in which the metal ion is associated with a charged or uncharged electron donor referred to as ligand. The ligand may be monodentate, bidentate or multidentate, that is, it may attach or co-ordinate using one or two or more donor atoms. Bidentate ligands form ring structures that include the metal ion and the two-ligand atoms attached to the metal. Their efficacy depends not solely on their affinity for the metal of interest but also on their affinity
Non-enzymatic antioxidants. Vitamin C, Vitamin C (ascorbic acid) is a very important, and powerful, antioxidant that works in aqueous environments of the body. Vitamin C cooperates with Vitamin E to regenerate α-tocopherol from α-tocopherol radicals in membranes and lipoproteins.

Ascorbic acid, behaves as a vinylogous carboxylic acid, wherein the double bond (“vinyl”) transmits electron pairs between the hydroxyl and the carbonyl. Ascorbate acts as an antioxidant by being available for energetically favorable oxidation. Reactive oxygen species oxidize (take electrons from) ascorbate first to monodehydroascorbate and then dehydroascorbate. The reactive oxygen species are reduced to water, while the oxidized forms of ascorbate are relatively stable and unreactive, and do not cause cellular damage.

A schematic diagram (Fig. 4) below shows the various forms of ascorbic acid (Vitamin C) and its reaction with radicals (R•). An ideal chelator should have high solubility in water, resistance to biotransformation, ability to reach site of metal storage, ability to retain chelating ability at the pH of body fluid and property of forming metal complexes that are less toxic than the free metal ion.

An ideal heavy metal chelator should be able to enter the cell easily, chelate the heavy metal from its complex with metallothionein or other proteins, and increase the excretion of the metal without its redistribution to other organs or tissues. Chelation therapy compromises with various side effects especially loss of essential elements. Chelating agents shows their binding affinity almost for all positively charged ions. Thus there is a need of safe and effective treatment against heavy metal toxicity. If any antioxidant satisfy above mentioned criteria of chelating agent it could serves as a chelating agent with less side effects.

Enzymatic antioxidants. One of the most effective intracellular enzymatic antioxidants is superoxide dismutase (SOD) which catalyzes the dismutation of O2•− to O2 with remarkably high reaction rates by successive oxidation and reduction of the transition metal ion and to the less-reactive species H2O2. Catalase is an enzyme present in the cells of plants, animals and aerobic bacteria. Catalase is located in a cell organelle called the peroxisome. The enzyme very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen.

Glutathione metabolism is one of the most essential of antioxidative defence mechanisms. GPx enzymes in presence of tripeptide glutathione (GSH) add two electrons to reduce peroxides. GPx decomposes peroxides to water (or alcohol) while simultaneously oxidizing GSH. The antioxidant properties of these enzymes allow them to eliminate peroxides as potential substrates for the Fenton reaction.

Figure 4. Conversion of ascorbic acid into different reduced forms at various pH indicating possible binding sites and free electrons responsible for their antioxidant and chelating property.

\[
\begin{align*}
2\text{H}_2\text{O}_2 & \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2 \\
2\text{GSH} + \text{H}_2\text{O}_2 & \xrightarrow{\text{GPx}} \text{GSSG} + 2\text{H}_2\text{O} \\
2\text{GSH} + \text{ROOH} & \xrightarrow{\text{GPx}} \text{GSSG} + \text{ROH} + \text{H}_2\text{O}
\end{align*}
\]

Non-enzymatic antioxidants. Vitamin C, Vitamin C (ascorbic acid) is a very important, and powerful, antioxidant that works in aqueous environments of the body. Vitamin C cooperates with Vitamin E to regenerate α-tocopherol from α-tocopherol radicals in membranes and lipoproteins.

Ascorbic acid, behaves as a vinylogous carboxylic acid, wherein the double bond (“vinyl”) transmits electron pairs between the hydroxyl and the carbonyl. Ascorbate acts as an antioxidant by being available for energetically favorable oxidation. Reactive oxygen species oxidize (take electrons from) ascorbate first to monodehydroascorbate and then dehydroascorbate. The reactive oxygen species are reduced to water, while the oxidized forms of ascorbate are relatively stable and unreactive, and do not cause cellular damage.

A schematic diagram (Fig. 4) below shows the various forms of ascorbic acid (Vitamin C) and its reaction with radicals (R•). Vitamin C scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that inhibits lipid peroxidation. Animal studies have suggested an antagonistic effect of ascorbic acid on lead absorption with its excellent chelating ability towards lead which is in good comparison to standard chelator ethylenediamine tetra acetic acid (EDTA). Chelation therapy compromises with various side effects especially loss of essential elements. Chelating agents shows their binding affinity almost for all positively charged ions. Thus there is a need of safe and effective treatment against heavy metal toxicity. If any antioxidant satisfy above mentioned criteria of chelating agent it could serves as a chelating agent with less side effects.

Metal chelation studies have shown that chelation of Zn(II) and Mn(II) ions takes place via two—OH groups while, Cd(II) ion binds via the O-3 atom only while the Hg(II) ion interaction leads to the oxidation of the ascorbic acid in aqueous solution. Vitamin C in plasma increases dose-dependently resistance to-lipid peroxidation, even in the presence of redox-active iron or copper and H2O2.
Antioxidants and metal toxicity

Vitamin C acts as a detoxifying agent by forming a poorly ionized but soluble complex. Thus Vitamin C supplementation, though observed in animal models, will have sustainable curative value among the already afflicted populations, neutralizing impact on freshly emerging metal poisoning scenario and possible proactive protection to those potentially susceptible to heavy metal exposure.

Vitamin E exists in eight different isomeric forms of two substructures tocopherol and tocotrienol (Fig. 5). Both structures are similar except the tocotrienol structure has double bonds on the isoprenoid units.

A consistent protective effect of Vitamin C has also been found in lung and colorectal cancer. In a recent published study, our group reported the remarkable effects of combined treatment of vitamin C and succimers like meso 2,3-dimercaptosuccinic acid (DMSA) or its monoisoamyl derivative (MiADMSA) on inhibited blood ALAD activity and in particular its beneficial effect in reducing the arsenic induced oxidative stress. Co-administration of vitamin C and MiADMSA in reducing liver and kidney arsenic burden supports the view that vitamin C acts as a detoxifying agent by forming a poorly ionized but soluble complex. Thus Vitamin C supplementation perspective, though observed in animal model, will have sustainable curative value among the already afflicted populations, neutralizing impact on freshly emerging metal poisoning scenario and possible proactive protection to those potentially susceptible to heavy metal exposure.

Vitamin E, Vitamin E exists in eight different isomeric forms of two substructures tocopherol and tocotrienol (Fig. 5). Both structures are similar except the tocotrienol structure has double bonds on the isoprenoid units.

α-tocopherol is the most active form of vitamin E in humans and is a powerful biological antioxidant which is considered to be the major membrane bound antioxidant employed by the cell. Its main antioxidant function is protection against lipid peroxidation. During the antioxidant reaction, α-tocopherol is converted to a α-tocopherol radical by the donation of labile hydrogen to a lipid or lipid peroxyl radical. The α-tocopherol radical can thus be reduced to the original α-tocopherol form by...
Vitamin E perform a unique function by interrupting free radical chain reactions via capturing the free radical. The free hydroxyl group on the aromatic ring is responsible for the antioxidant properties. The hydrogen from this group is donated to the free radical, resulting in a relatively stable free radical form of the vitamin.

The antioxidant function of this micronutrient enhances immunity by maintaining the functional and structural integrity of important immune cells. Vitamin E has the ability to prevent cell injury by maintaining the sulfhydryl groups of membrane proteins and by quenching free radicals.

Vitamin E has been reported to protect against arsenic toxicity. Protective value of vitamin E in arsenic induced toxicity could be attributed mainly to its antioxidant property or its location in the cell membrane and its ability to stabilize membrane by interacting with fatty acid chain. We reported increased antioxidant enzymes activities, and GSH concentration and decreased lipid per oxidation in animals co-administered with arsenic and vitamin E. Vitamin E may preserve cell membrane function including ion transport and membrane fluidity. It may also prevent the release of Fe²⁺ and Mg²⁺ from their binding proteins, potentially decreasing the rate of lipid per oxidation.

Our group has also reported beneficial effects of vitamins supplementation during lead intoxication. Intramuscular administration of vitamin E prevented inhibition of blood ALAD activity, elevation of urinary ALA excretion and was effective in reducing the lead induced altered biogenic amines levels in brain during the concomitant exposure lead. Vitamin E supplementation during concomitant lead exposure also prevented lead deposition in liver and blood. Some of the protective effects of vitamin E also emerge directly from its antioxidant property and some through its influence on the drug metabolising enzyme system. We also reported that administration of vitamin E when given in combination with meso 2,3-dimercaptosuccinic acid (DMSA) or its monoisoamyl derivative (MiADMSA) produced profound recoveries in sub-chronically lead exposed rat. Although, the group suggest that vitamin C was better in providing clinical recoveries than GSH but vitamin E from their oxidized forms. Since DHLA can neutralize free radicals it is known to regenerate Vitamin C which is even better than GSH and Vitamin E from their oxidized forms.

LA is readily absorbed from diet and is rapidly converted to Dihydrolipoic acid (DHLA) by NADH or NADPH in most tissues (Fig. 6). Studies have demonstrated superior anti-oxidant activity of DHLA as compared to LA. Since DHLA can neutralize free radicals it is known to regenerate Vitamin C which is even better than GSH and Vitamin E from their oxidized forms.

DHLA possess metal chelating properties which help the body to get rid of accumulated ingested toxins. It has been shown previously that oxidants may lead to cell death via lysosomal rupture and that this latter event may involve intralysosomal iron which catalyzes Fenton-type chemistry and resultant peroxidative damage to lysosomal membranes. LA stabilize lysosomes against oxidative stress, probably by chelating intralysosomal iron and, consequently, preventing intralysosomal Fenton reactions.

Packer et al. proposed a hypothesis of LA inducing cystine/cysteine uptake which examined the role of LA in stimulating GSH biosynthesis. In rats subjected to reperfusion injury following cerebral ischemia, LA restores brain GSH content and dramatically reduces the mortality rate from 78% to 26%.
LA has been reported to be effective in reducing the amount of OH\(^-\) generated by Fenton-type reactions and also a scavenger of peroxide and \(O_2^-\). Sadi et al. proposed the increase in level of SOD and catalase after incubating with LA in diabetic rats towards normal value. Antioxidant effects of LA is based on their interactions with peroxyl radicals, which are essential for the initiation of lipid peroxidation; and ascorbyl radicals of vitamin C. DHLA, can recycle ascorbyl radicals and reduce dehydroascorbate generated in the course of ascorbate oxidation by radicals. Therefore, DHLA may act as a strong chain-breaking antioxidant and may enhance the antioxidant potency of other antioxidants like vitamin C in both the aqueous and in hydrophobic membrane phase. LA is effective in vivo through suppression of glial reactivity. Since chronic reactive gliosis exacerbates diabetic neuropathy, the administration of LA can prevent neuropathy by reducing both oxidative stress and glial hyperactivity. Bhatt et al. have reported antioxidant potential (redox potential of LA/DHLA -320 mV) and greater efficacy of LA over captopril and quercetin against gallium arsenide (GaAs) induced oxidative stress in rats.

LA has long been known as an essential cofactor for mitochondrial bio-energetic enzymes. Various in vitro and in vivo studies suggest that LA also acts as a powerful micronutrient with diverse pharmacologic and antioxidant properties. Pharmacologically, LA improves glycemic control, polyneuropathies associated with diabetes mellitus, and effectively mitigates toxicities associated with heavy metal poisoning. As an antioxidant, LA directly terminates reactions with peroxyl radicals, which are essential for the initiation of lipid peroxidation; and ascorbyl radicals of vitamin C. DHLA, can recycle ascorbyl radicals and reduce dehydroascorbate generated in the course of ascorbate oxidation by radicals. Therefore, DHLA may act as a strong chain-breaking antioxidant and may enhance the antioxidant potency of other antioxidants like vitamin C in both the aqueous and in hydrophobic membrane phase. LA is effective in vivo through suppression of glial reactivity. Since chronic reactive gliosis exacerbates diabetic neuropathy, the administration of LA can prevent neuropathy by reducing both oxidative stress and glial hyperactivity. Bhatt et al. have reported antioxidant potential (redox potential of LA/DHLA -320 mV) and greater efficacy of LA over captopril and quercetin against gallium arsenide (GaAs) induced oxidative stress in rats.

LA has long been known as an essential cofactor for mitochondrial bio-energetic enzymes. Various in vitro and in vivo studies suggest that LA also acts as a powerful micronutrient with diverse pharmacologic and antioxidant properties. Pharmacologically, LA improves glycemic control, polyneuropathies associated with diabetes mellitus, and effectively mitigates toxicities associated with heavy metal poisoning. As an antioxidant, LA directly terminates reactions with peroxyl radicals, which are essential for the initiation of lipid peroxidation; and ascorbyl radicals of vitamin C. DHLA, can recycle ascorbyl radicals and reduce dehydroascorbate generated in the course of ascorbate oxidation by radicals. Therefore, DHLA may act as a strong chain-breaking antioxidant and may enhance the antioxidant potency of other antioxidants like vitamin C in both the aqueous and in hydrophobic membrane phase. LA is effective in vivo through suppression of glial reactivity. Since chronic reactive gliosis exacerbates diabetic neuropathy, the administration of LA can prevent neuropathy by reducing both oxidative stress and glial hyperactivity. Bhatt et al. have reported antioxidant potential (redox potential of LA/DHLA -320 mV) and greater efficacy of LA over captopril and quercetin against gallium arsenide (GaAs) induced oxidative stress in rats.

LA has long been known as an essential cofactor for mitochondrial bio-energetic enzymes. Various in vitro and in vivo studies suggest that LA also acts as a powerful micronutrient with diverse pharmacologic and antioxidant properties. Pharmacologically, LA improves glycemic control, polyneuropathies associated with diabetes mellitus, and effectively mitigates toxicities associated with heavy metal poisoning. As an antioxidant, LA directly terminates reactions with peroxyl radicals, which are essential for the initiation of lipid peroxidation; and ascorbyl radicals of vitamin C. DHLA, can recycle ascorbyl radicals and reduce dehydroascorbate generated in the course of ascorbate oxidation by radicals. Therefore, DHLA may act as a strong chain-breaking antioxidant and may enhance the antioxidant potency of other antioxidants like vitamin C in both the aqueous and in hydrophobic membrane phase. LA is effective in vivo through suppression of glial reactivity. Since chronic reactive gliosis exacerbates diabetic neuropathy, the administration of LA can prevent neuropathy by reducing both oxidative stress and glial hyperactivity. Bhatt et al. have reported antioxidant potential (redox potential of LA/DHLA -320 mV) and greater efficacy of LA over captopril and quercetin against gallium arsenide (GaAs) induced oxidative stress in rats.

LA has long been known as an essential cofactor for mitochondrial bio-energetic enzymes. Various in vitro and in vivo studies suggest that LA also acts as a powerful micronutrient with diverse pharmacologic and antioxidant properties. Pharmacologically, LA improves glycemic control, polyneuropathies associated with diabetes mellitus, and effectively mitigates toxicities associated with heavy metal poisoning. As an antioxidant, LA directly terminates reactions with peroxyl radicals, which are essential for the initiation of lipid peroxidation; and ascorbyl radicals of vitamin C. DHLA, can recycle ascorbyl radicals and reduce dehydroascorbate generated in the course of ascorbate oxidation by radicals. Therefore, DHLA may act as a strong chain-breaking antioxidant and may enhance the antioxidant potency of other antioxidants like vitamin C in both the aqueous and in hydrophobic membrane phase. LA is effective in vivo through suppression of glial reactivity. Since chronic reactive gliosis exacerbates diabetic neuropathy, the administration of LA can prevent neuropathy by reducing both oxidative stress and glial hyperactivity. Bhatt et al. have reported antioxidant potential (redox potential of LA/DHLA -320 mV) and greater efficacy of LA over captopril and quercetin against gallium arsenide (GaAs) induced oxidative stress in rats.
Antioxidants and metal toxicity

NAC pretreated animal effectively detoxifies or removes arsenic via GSH dependent pathway. It is probably due to an enhanced ability to maintain GSH homeostasis during exposure to toxic electrophiles generated by arsenic as well as its rapid elimination/excretion from the body.

Besides arsenic NAC shows chelating property against lead also. One of the first report by Pande et al. suggested that NAC could be used both as preventive as well as therapeutic agent along with MiADMSA/DMSA in the prevention or treatment of lead intoxication in rats. They reported that co-administration of NAC with DMSA reversed the altered ALAD and TBARS levels, increased the GSH level and decreased the lead level from blood and soft tissues. They proposed that NAC administration have a beneficial role, which is independent of chelation. However, no change in blood glutathione levels in lead exposed rats, as well as in NAC administered rats, provides a more complex theory that either all of the free sulfhydryl group may be utilized in complexing circulatory lead or perhaps the lead concentration in blood following exposure could not reach alarmingly high to influence blood GSH.

NAC pretreated animal effectively detoxifies or removes arsenic via GSH dependent pathway. It is probably due to an enhanced ability to maintain GSH homeostasis during exposure to toxic electrophiles generated by arsenic as well as its rapid elimination/excretion from the body.

Besides arsenic NAC shows chelating property against lead also. One of the first report by Pande et al. suggested that NAC could be used both as preventive as well as therapeutic agent along with MiADMSA/DMSA in the prevention or treatment of lead intoxication in rats. They reported that co-administration of NAC with DMSA reversed the altered ALAD and TBARS levels, increased the GSH level and decreased the lead level from blood and soft tissues. They proposed that NAC administration have a beneficial role, which is independent of chelation. However, no change in blood glutathione levels in lead exposed rats, as well as in NAC administered rats, provides a more complex theory that either all of the free sulfhydryl group may be utilized in complexing circulatory lead or perhaps the lead concentration in blood following exposure could not reach alarmingly high to influence blood GSH.

NAC pretreated animal effectively detoxifies or removes arsenic via GSH dependent pathway. It is probably due to an enhanced ability to maintain GSH homeostasis during exposure to toxic electrophiles generated by arsenic as well as its rapid elimination/excretion from the body.
Taurine. Taurine (2-aminoethanesulfonic acid) is a nonessential sulfur-containing amino acid that functions with glycine and gamma-amino butyric acid as a neuro inhibitory transmitter. The zwitterionic nature of taurine gives it high water solubility and low lipophilicity. Consequently compared with carboxylic amino acids, diffusion through lipophilic membranes is slow for taurine. It acts as a metabolic transmitter and additionally has a detoxifying effect and strengthens cardiac contractility. It crosses the blood brain barrier and has been implicated in a wide array of physiological phenomena including long term potentiation in the striatum/hippocampus membrane stabilization and protection against glutamate excitotoxicity. It also acts as an antioxidant and protects against toxicity of various heavy metals including arsenic, lead and cadmium. Zahorodnyi and Nebesna established that the sulfonate group of taurine is an electrophilic center and amide group is a nucleophilic center (Fig. 9). The biggest value of electrostatic potential is located around sulfonate group. This big value of energy of low occupied molecular orbital of taurine gives grounds to consider this substance as a reducing reagent and explains its antioxidative properties.

Low levels of taurine have been associated with retinal degeneration, growth retardation and cardiomyopathy. Taurine has also been used clinically in the treatment of cardiovascular diseases, hypercholesterolemia, seizure disorders, ocular disorders, diabetes, Alzheimer’s disease, hepatic disorders, cystic fibrosis and alcoholism. Taurine can act as a direct antioxidant by scavenging reactive oxygen species or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury. As a direct antioxidant, taurine is able to quench and detoxify some reactive intermediates such as hypochlorous acid generated by myeloperoxidase nitric oxide and H2O2. Taurine is a electrophilic center and amide group is a nucleophilic center. The biggest value of electrostatic potential is located around sulfonate group. This big value of energy of low occupied molecular orbital of taurine gives grounds to consider this substance as a reducing reagent and explains its antioxidative properties.

Antioxidants and metal toxicity

Flavonoids. Polyphenolic compounds constitute one of the most commonly occurring and ubiquitous groups of plant metabolites and represent an integral part of human. Their common structural feature is the diphenylpropane moiety, which consists of two aromatic rings linked through three carbon atoms that together usually form an oxygenated heterocycle. Phenolic compounds acting as antioxidants may function as terminators of free radical chains and as chelators of redox-active metal ions that are capable of catalyzing lipid peroxidation. One of the most actively studied properties of flavonoids is their protection against oxidative stress. For example, flavonoids are ideal scavengers of peroxyl radicals due to their favorable reduction potentials relative to alkyl peroxyl radicals and thus, in principle, they are effective inhibitors of lipid peroxidation. Of particular importance is the hydrogen (electron) donating ability of a flavonoid molecule which acts to scavenge a reactive radical species, and is primarily associated with the presence of a B-ring catechol group (dihydroxylated B-ring). One important structural feature which is partly responsible for the antioxidant properties of flavonoids involves the presence of 2,3 unsaturation in conjugation with a 4-oxo group in the C-ring. In addition, the presence of functional groups involving both hydroxyl groups of ring-B and the 5-hydroxy group of ring-A are all important contributors in the ability of flavonoids to chelate redox-active metals and thus prevent catalytic breakdown of hydrogen peroxide (Fenton chemistry).
Antioxidants and metal toxicity

The propensity of a flavonoid to inhibit free-radical mediated events is governed by its chemical structure. Multiple hydroxyl groups confer upon the molecule substantial antioxidant, and chelating ability. A double bond and carbonyl function in the heterocycle or polymerization of the nuclear structure increases activity by affording a more stable flavonoid radical through conjugation and electron delocalization. Quercetin has the ability to form semiquinone and quinone type compounds by undergoing one or two electron oxidation respectively. In this form it is able to chelate metal ions and form five member chelating ring. Its anti-radical property is directed to scavenge \( \cdot \mathrm{OH} \) and the superoxide anion, highly reactive species implicated in the initiation of lipid peroxidation. On the other hand, quercetin as a phenolic compound, may act as a metal-chelating agent, and in fact, it belongs to a special class of bidentate O,O-coordinating ligands which is capable of undergoing both one or two electron oxidations, forming semiquinone and quinone type compounds respectively. There are many reports in the literature of quercetin-metal complexes but most of these are solution studies in which no compounds were isolated. Bravo and Anacona, have reported coordination site at quercetin and its bonding properties on the basis of spectroscopic analysis. They proposed that carbonyl oxygen atom does not participate in coordination to the metal ions and coordination proceeds through the ortho-phenolic groups located on the B ring (Fig. 10). Their ability to form complexes with some p-, d- and f-electron metals makes them interesting analytical reagents. Quercetin possesses three possible chelating sites in competition: the 3-hydroxychromone, the 5-hydroxychromone and the 3',4'-dihydroxyl groups and is most widely used for detection of metals bound to flavonoid ligands owing to their highly sensitive molecular fluorescence properties. Analytical procedures have been developed for Al, Cr, W, Zr, Ti, Fe, Mo, Zr, Hf, Ge, Ru, Pd, Os, Pt and Au. Quercetin contains numerous double bonds and hydroxyl groups that can donate electrons through resonance to stabilize the free radicals. The radical scavenging properties associated with the structure of quercetin defend against oxidative stress and in doing so, reduce heart disease, prevent cancer, and slow the aging atom chain (structure A). The chemical structure of flavonoids are based on a C15 skeleton with a chromane ring bearing a second aromatic ring B in position 2, 3 or 4 (structure B).

Various subgroups of flavonoids are classified according to the substitution patterns of ring C. Both the oxidation state of the heterocyclic ring and the position of ring B are important in the classification.

The flavonol quercetin (3',3,4',5,7-pentahydroxyflavone) is one of the most abundant dietary flavonoids. It is found in many fruits and vegetables, as well as olive oil, red wine and tea. A recent report indicates that blood plasma concentrations may reach in excess of 20 \( \mu \)M quercetin and quercetin metabolites in response to quercetin-rich diets. Quercetin was found to scavenge free radicals and reduce the oxidability and cytotoxic effects of low density lipoproteins. High consumption of flavonoid rich food has therefore also been linked to a reduced incidence of cancers at various sites. Quercetin have multiple biological, pharmacological and medicinal properties including anti-inflammatory, anti-allergic, antiviral, anti-thrombotic, anti-mutagenic, antineoplastic and cytoprotective effects. Various epidemiological and dietary studies suggest that quercetin may play a useful role in preventing neurodegeneration, especially age-related cognitive, motor and mood decline and protect against oxidative stress as well as cerebral ischemic injuries. Quercetin induces growth inhibition and cell death in a variety of cancer cells including glioma cells. Quercetin has been reported to have both protective and detrimental effects. The precise molecular mechanism of quercetin action is poorly understood. Quercetin might exert the protective effect against the cell death associated with generation of ROS.

The flavonol quercetin (3',3,4',5,7-pentahydroxyflavone) is one of the most abundant dietary flavonoids. It is found in many fruits and vegetables, as well as olive oil, red wine and tea. A recent report indicates that blood plasma concentrations may reach in excess of 20 \( \mu \)M quercetin and quercetin metabolites in response to quercetin-rich diets. Quercetin was found to scavenge free radicals and reduce the oxidability and cytotoxic effects of low density lipoproteins. High consumption of flavonoid rich food has therefore also been linked to a reduced incidence of cancers at various sites. Quercetin have multiple biological, pharmacological and medicinal properties including anti-inflammatory, anti-allergic, antiviral, anti-thrombotic, anti-mutagenic, antineoplastic and cytoprotective effects. Various epidemiological and dietary studies suggest that quercetin may play a useful role in preventing neurodegeneration, especially age-related cognitive, motor and mood decline and protect against oxidative stress as well as cerebral ischemic injuries. Quercetin induces growth inhibition and cell death in a variety of cancer cells including glioma cells. Quercetin has been reported to have both protective and detrimental effects. The precise molecular mechanism of quercetin action is poorly understood. Quercetin might exert the protective effect against the cell death associated with generation of ROS.

**Figure 10.** Structure of quercetin showing coordination through the ortho-phenolic groups located on the B ring.
processes in cells responsible for degenerative diseases. Quercetin interferes by reacting with the radicals formed in the process of lipid peroxidation. Quercetin does not only stop the propagation of lipid peroxidation, but also increases glutathione (GSH) levels. GSH can convert hydrogen peroxide to oxygen and water, preventing the formation of free radicals. Oxidative stress can cause cell death by means of prolonged elevations of intracellular Ca\(^{2+}\) concentrations. Quercetin can protect cells suffering oxidative stress and thus prevent Ca\(^{2+}\)-dependent cell death.

The oxidation of low-density lipoproteins (LDL) can result in the formation of atherosclerotic plaques, leading to cardiovascular disease. Quercetin has displayed the ability to prevent the oxidation of LDL by scavenging free radicals and chelating transition metal ions. Graf and co-workers found a 21% reduction in cardiovascular disease mortality when the intake of quercetin was greater than 4 mg/day. Quercetin when reacts with a free radical, it donates a proton and becomes a radical itself, but the resulting unpaired electron is delocalized by resonance, making the quercetin radical too low in energy to be reactive. Three structural groups aid in quercetin's ability to maintain its stability and act as an antioxidant when reacting with free radicals: the B ring o-dihydroxyl groups, the 4-oxo group in conjugation with the 2,3-alkene, and the 3- and 5-hydroxyl groups. The functional groups can donate electrons to the rings, which increase the number of resonance forms available in addition to those created by the benzene structure.

Many flavonoids are bound to sugars in their natural state, the O-glycoside form, where glycosylation can occur at any hydroxyl group to yield a sugar. The most common quercetin glycosides have a sugar group at the 3-position, such as quercetin-3-O-β-glucoside shown in Figure 11.

Glycosylated structures are most common in nature, not the parent compound. Most studies assessing the antioxidant properties of quercetin utilize the parent form; however, analysis of plasma after quercetin consumption indicates that quercetin metabolites, like glucuronide (quercetin-3-O-β-D-glucuronide), are the primary compounds circulating in the blood. Quercetin can also protect against the more obvious environmental causes of free radicals, such as smoking. Cigarette tar is a source of free radicals, which has been found to damage erythrocyte membranes. Begum and Terao found that the quercetin and its conjugate metabolites (quercetin-3-O-β-glucuronide and quercetin-3-O-β-glucoside) could protect erythrocytes from the membranous damage that is caused by smoking. The control used in the study was flavone, which has the basic structure of quercetin but no hydroxyl groups, and it had no effect on the erythrocytes. This indicated that the hydroxyl groups are important to the antioxidant properties of quercetin.

Quercetin is also reported to prevent apoptosis in several cells such as fibroblasts, cardiomyoblasts, and epithelial cells. Quercetin was found to attenuate oxidative damage induced by arsenic by restoring GSH contents and ROS levels and reducing TBARS levels. Quercetin has also been reported to increase metallothionein expression and prevent cadmium-induced Nephrotoxicity. In spite of these positive effects of quercetin, there have been a number of conflicting report about quercetin like it acts as a pro-oxidant or has some moderate toxicity however, it is widely accepted that the beneficial effects of quercetin are due mainly to its antioxidant properties and also due to regulation of signaling pathway. Quercetin administration was also found to be associated with reduced condition of oxidative stress induced by GaAs exposure. Mishra et al. have also reported that the combined treatment with quercetin and MiADMSA was not only able to chelate arsenic from the cell but also ameliorate oxidant levels, i.e., abatement of toxic effects of arsenic.

Garlic, Garlic (Allium sativum L., family Liliaceae), called Lasan in India, is a medicinal plant which has been used for thousands of years in Indian Ayurvedic medicine. It is also used with spices to give a special flavor and fragrance to the food. Garlic contains a number of organosulfur compounds which are widely believed to be the active agents. The major medicinal compound obtained from garlic is Allicin, a powerful anti-biotic and anti-fungal agent. Precursor of Allicin is alliin which on decomposition with pyruvic acid and ammonia in the presence of garlic constitutional enzyme...
Alliinase gives Allicin. Whenever any part of the garlic is damaged, Allicin is formed which has the characteristic odor of garlic.

The major sulfur-containing compounds in intact garlic are γ-glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides (alliin). Both are abundant as sulfur compounds, and alliin is the primary odorless, sulfur-containing amino acid, a precursor of Allicin, methiin, (+)-S-(trans-1-propenyl)-L-cysteine sulfoxide, and cycloalliin.195 These sulfoxides, except cycloalliin, are converted into thiosulfonates through enzyme reactions when raw garlic is cut or crushed. Besides this, a number of other antibiotic principles have also been isolated, namely, methyl-allyl thiosulfonates, 1-propenyl allyl thiosulfinate, L-glutamyl-S-allyl-L-cysteine, S-allyl mercaptocysteine, diallyl disulfide, -trisulfide, allyl methyl trisulfide, -disulfide, diallyl tetra sulfide, allyl methyl tetra sulfide, Dimethyl trisulfide, Diallyl sulfide, 2-vinyl-4-H,1,3-di-thiin, 3-vinyl-4-H,1,2-dithiin, E-ajoene, Z-ajoene, Allyl mercaptan.196 Structures of some important constituents of garlic are illustrated in Figure 12.

According to the recent pharmacological findings, garlic is a preventive rather than therapeutic. The pharmacological effects of garlic have mostly been attributed to its hypoglycemic198 hypolipidimic199 anticoagulant, antihypertensive200 antihematotoxic, anticancer, immune system modulatory, antiatherosclerotic, antioxidant properties.196,197 In addition to this, the bulb of garlic is used as an antirheumatic and stimulant beside its use in conditions like paralysis, forgetfulness, tumor colicky pain and chronic fever.201 Recent studies have demonstrated that garlic exerts its therapeutic effect by increasing nitric oxide (NO) production.202,203 It is also found to have free radical scavenging action and inhibits oxidative modification of low-density lipoproteins.204 The intrinsic antioxidant activity of garlic, aged garlic extracts (AGE) and some garlic constituents have been widely documented in vivo and in vitro.205 LDL oxidation has been recognized as playing an important role in the initiation and progression of atherosclerosis. Popov and Lewin206 observed the antioxidant effect of the aqueous extract of intact garlic. They determined that sulphur bearing components of aqueous garlic extract are the presence of -SH/-OH group either in the parent molecule or in their reduced form. It suggest that if an antioxidant molecule is able to provide free electron either in the form of a negative charge or in the form of a lone pair of electrons it may provide a chelating site for toxic metal. Besides, providing beneficial effects in eliminating heavy metal body burden and thereby reversing the altered biochemical variables these antioxidants could be useful in enhancing endogenous antioxidant levels. These antioxidants may also be supplemented during chelation therapy with a thiol chelator to get optimum therapeutic effects with fewer side effects.223,224

Conclusion

This review provides an insight on the beneficial effects of different antioxidants in preventing arsenic or lead body burden and oxidative stress. The main structural features in antioxidants are the presence of -SH/-OH group either in the parent molecule or in their reduced form. It suggest that if an antioxidant molecule is able to provide free electron either in the form of a negative charge or in the form of a lone pair of electrons it may provide a chelating site for toxic metal. Besides, providing beneficial effects in eliminating heavy metal body burden and thereby reversing the altered biochemical variables these antioxidants could be useful in enhancing endogenous antioxidant levels. These antioxidants may also be supplemented during chelation therapy with a thiol chelator to get optimum therapeutic effects with fewer side effects.223,224

Acknowledgements

Author thanks Dr. R. Vijayaraghavan, Director of the establishment for his support and Dr. Abhishek Yadav for his help during the preparation of this manuscript.

References

1. Bargagli R. Trace metals in Antarctica related to climate change and increasing human impact. Rev Environ Contam Toxicol 2000; 166:129-73.
2. Mishra D, Mehta A, Flora SJS. Reversal of hepatic apoptosis with combined administration of DMSA and its analogues in guinea pigs: role of glutathione and linked enzymes. Chem Res Toxicol 2008; 21:400-7.
3. Flora SJS, Saxena G, Mehta A. Reversal of lead-induced neuronal apoptosis by chelation treatment in rats: role of ROS and intracellular Ca²⁺. J Pharmacol Exp Ther 2007; 322:108-16.

4. Watanabe M, Henki K, Ogawa K, Suzuki T. Cadmium dependent generation of reactive oxygen species and mitochondrial DNA breaks in photosynthetic and non-photosynthetic strains of Euglena gracilis. Comp Biochem Physiol Toxicol Pharmacol 2003; 134:227-34.

5. Delhi AD, Paine AJ. Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000. Hum Exp Toxicol 2001; 20:439-51.

6. Lee YW, Ha MS, Kim YK. Role of reactive oxygen species and glutathione in inorganic mercury-induced injury in human glioma cells. Neurochem Res 2001; 26:1163-82.

7. Halliwell B. Antioxidants in human health and disease. Antioxid Redox Signal 1996; 16:33-50.

8. Gutteridge JM, Halliwell B. Iron toxicity and oxygen radicals, Ballieres Clin Haematol 1989; 2:195-256.

9. Deiderer A, Falcois M. Prokaryotic Cu, Zn superoxidase dismutase. Biochem Soc Trans 2003; 31:1323-5.

10. Hlavaty JJ, Benner JS, Hornstra LJ, Schildkraut I. Identification of the metal-binding sites of restriction endonucleases by Fe³⁺ mediated oxidant stress. Biochemistry 2000; 39:3097-105.

11. Wiseman H. Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. Biochem J 1996; 313:17-29.

12. Desideri A, Falconi M. Prokaryotic Cu, Zn superoxidase dismutase. Biochem Soc Trans 2003; 31:1323-5.

13. Leonard S, Gannett PM, Rojanasakul Y, Schwegler-Berry D, Castranova V, Vallyathan V, Brewer GJ, Dick RD, Grover DK, LeClaire V, Tseng M, Wicha M, et al. Treatment of metastatic cancer with tetrathiomolybdate, an anticopper, antiangiogenic agent: Phase I study. Clin Cancer Res 2000; 6:1-10.

14. Simon JA, Hudes ES. Relationships of ascorbic acid to blood lead levels. J Am Med Assoc 1989; 260:7-12.

15. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Rad Biol Med 1999; 26:1034-53.

16. Mates JM, Perez-Gomez C, De Castro IN. Antioxidant enzymes and human diseases. Clin Chem 1999; 35:595-603.

17. Anderson O. Principles and recent developments in chelation treatment of metal intoxication. Chem Rev 1999; 99:2683-710.

18. Jin MM, Cherian MG. The search for chelate antagonists for chronic cadmium intoxication. Toxicology 1990; 62:1-25.

19. McLeod JL, Fridovich I. Superoxide dismutase as an enzyme function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244:6049-55.

20. Hsuan-Yu C, Sung-Liang Y, Linzhao C, Pan-Chyr Y, Chi VD. Arsenic induced by dimethylarsinic acid, a metabolite of inorganic arsenics, are strongly

21. Applegate LA, Luscher P, Tyrrell RM. Induction of heme oxygenase: a general response to oxidative stress in shock and inflammation. Curr Med Chem 2004; 11:1047-62.

22. Cooke MS, Evans MD, Diwan LA. Mechanisms underlying arsenic carcinogenesis: Part I. Arch Environ Contam Toxicol 2005; 49:119-23.

23. Pi J, Horiguchi S, Sun Y, Nikaido M, Shimojo N, Hayashi T. A potential mechanism for inhibition of the L-kynurenine pathway by lead. J Inorg Biochem 1998; 70:239-44.

24. Bechara EJH, Abdalla DSP. Free radicals involvement in neurological diseases. Arch Environ Contam Toxicol 1997; 33:31-8.

25. Waalkes MP, Liu J, Ward JM, Diwan LA. Mechanisms underlying arsenic carcinogenesis: Part II. Arch Environ Contam Toxicol 2005; 49:119-23.

26. Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mut Res-Fund Mol Genet 1989; 205:165-76.

27. Cuzzorcrea S, Thiemermann C, Salvemini D. Potential therapeutic effect of antioxidant peptides and metal chelators on cell death in the rat. Life Sci 1999; 64:1057-63.

28. Monrotero JP, Bechara EJH, Abdalla DSP. Free radicals involvement in neurological disorders and lead poisoning. Mol Cell Biochem 1991; 103:73-83.

29. Theriault RJ, Rueckl J, Hwang J, Kaczmarek L, Hsu P, Chong A. Lead and selenium levels in the blood of children. J Am Ind Med Assoc 2001; 22:1082-9.

30. Netto AD, Paine AJ. Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000. Hum Exp Toxicol 2001; 20:439-51.

31. Halliwell B. Antioxidants in human health and disease. Antioxid Redox Signal 1996; 16:33-50.

32. Gutteridge JM, Halliwell B. Iron toxicity and oxygen radicals, Ballieres Clin Haematol 1989; 2:195-256.

33. Desideri A, Falconi M. Prokaryotic Cu, Zn superoxidase dismutase. Biochem Soc Trans 2003; 31:1323-5.

34. Hlavaty JJ, Benner JS, Hornstra LJ, Schildkraut I. Identification of the metal-binding sites of restriction endonucleases by Fe³⁺ mediated oxidant stress. Biochemistry 2000; 39:3097-105.

35. Wiseman H. Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. Biochem J 1996; 313:17-29.

36. Pi J, Horiguchi S, Sun Y, Nikaido M, Shimojo N, Hayashi T. A potential mechanism for inhibition of the L-kynurenine pathway by lead. J Inorg Biochem 1998; 70:239-44.

37. Applegate LA, Luscher P, Tyrrell RM. Induction of heme oxygenase: a general response to oxidative stress in shock and inflammation. Curr Med Chem 2004; 11:1047-62.

38. Cooke MS, Evans MD, Diwan LA. Mechanisms underlying arsenic carcinogenesis: Part I. Arch Environ Contam Toxicol 2005; 49:119-23.
71. Kojo S, Vitamin C: basic metabolism and its function as an index of oxidative stress. Curr Med Chem 2001; 8:101-106.

72. Arita M, Sato Y, Miyata A, Tanabe T, Takahashi E, Kaydend HJ, et al. Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem J 1995; 306:437-43.

73. Basu TK, Dickerson JW. Vitamin E: In: Vitamins in human health and disease. Wallingford UK: CAB International 1996; 214-27.

74. Lee TC, Ho RC. Modulation of cellular antioxidant defense activities by sodium arsenate in human fibroblasts. Arch Toxicol 1994; 69:498-504.

75. Flora SJS, Pande M, Mehta A. Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. Chem Biol Interact 2003; 145:267-80.

76. Mittal M, Flora SJS. Vitamin E protects oxidative stress and essential metal imbalance during concomitant exposure to arsenic and fluoride in male mice. Drug Chem Toxicol 2007; 30:263-81.

77. Ganther HE. Modification of methyl mercury toxicity and metabolism by selenium and vitamin E: possible mechanisms. Environ Health Perspect 1978; 25:71-6.

78. Abubakar MG, Taylor A, Ferns GA. Regional accumulation of aluminum in the rat brain is affected by dietary vitamin E. J Trace Elem Med Biol 2004; 18:53-9.

79. Dhawan M, Flora SJS, Tandon SK. Preventive and therapeutic role of vitamin E in chronic Plumbism. Biol Environ 1989; 2:335-41.

80. Weber P, Bendiach A, Machlin LJ. Vitamin E and human health: rationale for determining recommended intake levels. Nutrition 1997; 13:450-60.

81. Flora SJS, Pande M, Mehta A. Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. Chem Biol Interact 2003; 145:267-80.

82. White E, Shannon JS, Patterson RE. Relationship between vitamin and calcium supplement use and colon cancer. Cancer Epidemiol Biomark 1997; 6:769-74.

83. Greenberg ER, Baron JA, Tosteson TD, Freeman DH, Beck GJ, Bond JH. Clinical-trial of antioxidant vitamins to prevent colorectal adenoma. N Engl J Med 1994; 331:141-7.

84. Macela R, Du Benedetto R, Vai R, Filesi G, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem 2005; 16:577-86.

85. Sarouli H, Hogg N, Frejaville C, Takahashi E, Kayden HJ, et al. Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem J 1995; 306:437-43.

86. Karoui H, Hogg N, Frejaville C, Takahashi E, Kayden HJ, et al. Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem J 1995; 306:437-43.

87. Le C, Ho RC. Modulation of cellular antioxidant defense activities by sodium arsenate in human fibroblasts. Arch Toxicol 1994; 69:498-504.

88. Flora SJS, Pande M, Mehta A. Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. Chem Biol Interact 2003; 145:267-80.

89. Mittal M, Flora SJS. Vitamin E protects oxidative stress and essential metal imbalance during concomitant exposure to arsenic and fluoride in male mice. Drug Chem Toxicol 2007; 30:263-81.

90. Ganther HE. Modification of methyl mercury toxicity and metabolism by selenium and vitamin E: possible mechanisms. Environ Health Perspect 1978; 25:71-6.

91. Abubakar MG, Taylor A, Ferns GA. Regional accumulation of aluminum in the rat brain is affected by dietary vitamin E. J Trace Elem Med Biol 2004; 18:53-9.

92. Dhawan M, Flora SJS, Tandon SK. Preventive and therapeutic role of vitamin E in chronic Plumbism. Biol Environ 1989; 2:335-41.

93. Greenberg ER, Baron JA, Tosteson TD, Freeman DH, Beck GJ, Bond JH. Clinical-trial of antioxidant vitamins to prevent colorectal adenoma. N Engl J Med 1994; 331:141-7.

94. Macela R, Du Benedetto R, Vai R, Filesi G, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem 2005; 16:577-86.

95. Sarouli H, Hogg N, Frejaville C, Takahashi E, Kayden HJ, et al. Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem J 1995; 306:437-43.

96. Le C, Ho RC. Modulation of cellular antioxidant defense activities by sodium arsenate in human fibroblasts. Arch Toxicol 1994; 69:498-504.

97. Le C, Ho RC. Modulation of cellular antioxidant defense activities by sodium arsenate in human fibroblasts. Arch Toxicol 1994; 69:498-504.

98. Le C, Ho RC. Modulation of cellular antioxidant defense activities by sodium arsenate in human fibroblasts. Arch Toxicol 1994; 69:498-504.

99. Le C, Ho RC. Modulation of cellular antioxidant defense activities by sodium arsenate in human fibroblasts. Arch Toxicol 1994; 69:498-504.

100. Le C, Ho RC. Modulation of cellular antioxidant defense activities by sodium arsenate in human fibroblasts. Arch Toxicol 1994; 69:498-504.
Antioxidants and metal toxicity

130. Flora SJS, Tripathi N, Kannan GM. Arsenic induced biochemical alterations and their reversibility following chelation therapy in rats. Poisoning control and Environ Toxicol 2000; 29-37.

131. Brakenhoff JP, Commandeur JN, Wormhout LW, Groot EJ, Vermeulen NP. Molecular mechanism of toxic effects of fomepizole in rat hepatocytes and subcellular rat liver fractions. Carcinogenesis 1996; 17:175-24.

132. Vermeulen NP, Commandeur JN, Groot EJ, Wormhout LW, Ramrathshing S, Li OJ, Brakenhoff JP. Toxicity of fomepizole in rat hepatocytes and mechanism-based protection against it. Chem Biol Interact 1998; 110:35-49.

133. Olive MF. Interactions between taurine and ethanol in the central nervous system. Amino Acids 2002; 23:345-57.

134. Tsuji A, Tama I. Sodium- and chloride-dependent transport of taurine at the blood-brain barrier. Adv Exp Med Biol 1996; 403:385-91.

135. Salminen J, Soinila G, Piekkanen TP, Ruohonen N, Ahre L. The effects of systemically administered taurine and N-pivaloyltaurine on striatal extracellular dopamine and taurine in freely moving rats. Naunyn-Schmiedeberg's Archives of Pharmacology 2003; 368:134-41.

136. Dominy J Jr, Thinschmidt JS, Perij J, Dawson R Jr, Papke RL. Taurine-induced long-lasting potentiation in the rat hippocampus shows a partial dissociation from total hippocampal taurine content and independence from activation of known taurine transporters. Journal of Neurochemistry 2004; 89:1195-205.

137. Birdwall TC. Therapeutic applications of taurine. Alt Med Rev 1998; 3:128-36.

138. Olive MF. Interactions between taurine and ethanol in the central nervous system. Amino Acids 2002; 23:345-57.

139. Schroeter H, Boyd C, Spencer JP, Williams RJ, Cadenas E, Rice-Evans C. MAPK signal-ling in neurodegeneration: influences of flavonoids and of nitric oxide. Neurobiol Aging 2002; 23:861-80.

140. Bouktaib M. Regio- and stereoselective synthesis of the major metabolite of quercetin, 3-O-pivaloylquercetin. J Med Food 2005; 8:281-90.

141. Mariani C. Flavonoid characterization and in vitro antioxidant activity of Aconitum. J Agric Food Chem 2002; 50:5338-42.

142. Lee K, Kim Y, Kim D. Major phenolics in apple and their contribution to the total antioxidant capacity. J Agric Food Chem 2003; 51:6516-20.

143. Kuntic V, Blagojevic S, Malesev D, Radovic Z, Bogavac M. Spectrophotometric Investigation of the Pd(II)-Quercetin Complex in 50% Ethanol. Chemical 1998; 129:41-8.

144. Heim KE, Tagliaferro AR, Bohily DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002; 13:572-84.

145. Mariani C. Flavonoid characterization and in vitro antioxidant activity of Aconitum. J Agric Food Chem 2002; 50:5338-42.

146. Schroeter H, Boyd C, Spencer JP, Williams RJ, Cadenas E, Rice-Evans C. MAPK signaling in neurodegeneration: influences of flavonoids and of nitric oxide. Neurobiol Aging 2002; 23:861-80.

147. Mariani C. Flavonoid characterization and in vitro antioxidant activity of Aconitum. J Agric Food Chem 2002; 50:5338-42.

148. Mariani C. Flavonoid characterization and in vitro antioxidant activity of Aconitum. J Agric Food Chem 2002; 50:5338-42.
Antioxidants and metal toxicity

194. Agarwal RC. Therapeutic action of garlic constituents. Med Res Rev 1996; 16:111-24.
195. Fujimura M, Yoshimura M, Tsuno S, Murakami F. "Allithiamine," a newly found derivative of vitamin B1 IV. on the alliin homologues in the vegetables. J Biochem 1958; 45:141-9.
196. Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. Phtyrore 2003; 17:97-106.
197. Amagase H. Clarifying the real bioactive constituents of garlic. J Nutr 2006; 136:716-25.
198. Augusti KT, Sheela CG. The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochim Biophys Acta 1998; 1379:233-44.
199. Ismail MF, Gad MZ, Hamdy MA. Study of the hypolipidemic properties of pectin, garlic and ginseng in hypercholesterolemic rabbits. Pharmacol Res 1999; 39:157-66.
200. Mashour NH, Lin GL, Frishman WH. Herbal medicine for the treatment of cardiovascular disease: clinical considerations. Arch Intern Med 1998; 9:2225-34.
201. Nadkarni AK. Indian Materia Medica I and II. Popular Prakashan, Bombay 1992; 66-71.
202. Maslin CA, Brown ID, Xia-Hua Z. Nitric oxide a mediator of garlic action? Biochem Soc Transact 1997; 25:408.
203. Sooranna SR, Hirani J, Das I. Garlic can induce both GTP cyclohydrolase and nitric oxide synthase activity in choriocarcinoma cells. Biochem Soc Trans 1995; 23:543.
204. Nagatoshi I, Audowin BN, Benjamin HSL. Aged garlic extract and its constituents inhibit Cu2+-induced oxidative modification of low-density lipoproteins. Planta Medica 1997; 63:263-4.
205. Rabinkov A, Miron T, Konstantinovski I, Wilchek M, Mirelman D, Weiner L. The mode of action of allin: trapping of radicals and interaction with thiol containing proteins. Biochim Biophys Acta 1997; 1995; 1379:233-44.
206. Iqbal M, Athar M. Attenuation of Iron-Nitrilotriacetate (Fe-NTA)-mediated renal oxidative stress, toxicity and hyperproliferative response by prophylactic treatment of rats with garlic oil. Food Chem Toxicol 1998; 36:485-95.
207. Popov I, Lewin G. Antioxidant effects of aqueous garlic extract. 2nd communication: inhibition of the Cu(2+)-initiated oxidation of low density lipoproteins. Arzneimittel Forschung 1994; 44:604-7.
208. Geng S, Lau BH. Aged garlic extract modulates glutathione redox cycle and superoxide dismutase activity in vascular endothelial cells. Phytother Res 1997; 11:54-66.
209. Wei Z, Lau BHS. Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. Nutr Res 1998; 18:61-70.
210. Numagami Y, Sato S, Ohnishi T. Attenuation of rat ischemic brain damage by aged garlic extracts: a possible protecting mechanism as antioxidants. Neurochem Int 1996; 29:35-45.
211. Yamasaki T, Lau BHS. Garlic compounds protect vascular endothelial cells from oxidant injury. Folia Pharmacol Jpn 1997; 110:138-41.
212. Geng Z, Rong Y, Lau BH. S-allyl cysteine inhibits activation of nuclear factor kappaB in human T-cells. Free Radic Biol Med 1997; 23:345-50.
213. Oommen S, Anno RJ, Srinivas G, Karunagaran D. Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. Europ J Pharmacol 2004; 485:97-103.
214. Horie T, Murayama T, Mohima T, Ishi F, Minamisada Y, Fueva T, Awasu S. Protection of liver microsomal membranes from lipid peroxidation by garlic extract. Planta Med 1989; 55:506-8.
215. Senapati SK, Dey S, Dwivedi SK, Swarup D. Effects of garlic (Allium sativum L.) on tissue lead levels in rats. J Ethnopharmacol 2001; 76:229-32.
216. Chowdhury AR, Das T, Sharma A, Talukder D. Use of crude extract of garlic (Allium sativum L.) in reducing cytotoxic effects of arsenic in mouse bone marrow. Phytother Res 1993; 7:163-6.
217. Chowdhury AR, Das T, Sharma A, Talukder K. Dietary garlic extract in modifying clastogenic effects of inorganic arsenic in mice: two generation studies. Mut Res 1996; 359:165-70.
218. Flora SJS, Bhart K, Mehta A. Arsenic moiety in gallium arsenide is responsible for neuronal apoptosis and behavioral alterations in rats. Toxicol Appl Pharmacol 2009; In press.