

## Review

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

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**Abbreviations:** DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ROS, reactive oxygen species; RNS, reactive nitrogen species; NO, nitric oxide; ALAD,  $\delta$ -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,  $\alpha$ -lipoic acid; DHLA, dihydrolipoic acid; PUFA, poly unsaturated fatty acids; AA, ascorbic acid; DHAA, dihydroascorbic acid; NAC, N-acetyl-L-cysteine

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

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,  $\alpha$ -lipoic acid, melatonin, 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.<sup>1</sup> Numerous studies have reported toxic and carcinogenic effects induced when humans and animals are exposed to certain metals especially arsenic,<sup>2</sup> lead,<sup>3</sup> cadmium,<sup>4</sup> chromium<sup>5</sup> and mercury.<sup>6</sup> 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).<sup>7</sup> 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.<sup>8</sup> ROS at higher concentration are important mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids (termed oxidative stress).<sup>9</sup> The harmful effects of ROS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes.<sup>10</sup>

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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.

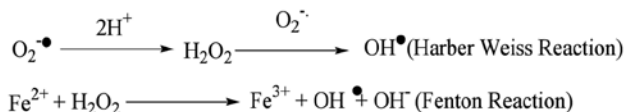
## Chemistry and Biochemistry of Free Radicals

**Reactions of free radicals.** Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons.<sup>11</sup> 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 ( $O_2^{\bullet-}$ ). Superoxide ion is rapidly detoxified initially to hydrogen peroxide because of its dismutation reaction to form hydrogen peroxide and oxygen<sup>12</sup> and finally to water by Cu, Zn-SOD and/or Mn-SOD.



Significant amounts of hydrogen peroxide are formed in the mitochondria and this is enriched with antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) which minimizes the oxidative stress.<sup>13</sup>



Fe (II) can participate in the Fenton reaction, generating highly reactive hydroxyl radical. Thus under stress conditions  $O_2^{\bullet-}$  facilitates  $OH^{\bullet}$  production from  $H_2O_2$  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.

## 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,

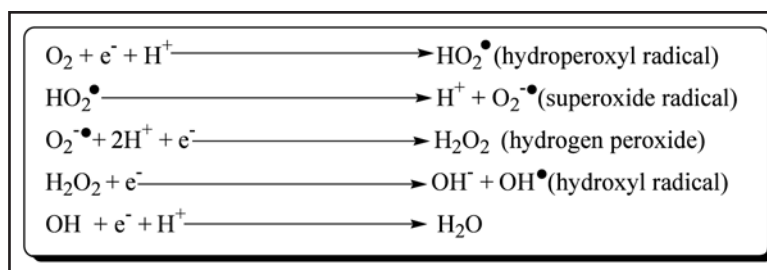


Figure 1. Redox reaction showing generation of various Reactive Oxygen Species (ROS).

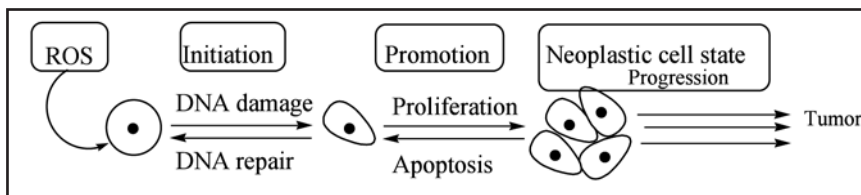


Figure 2. Effects of ROS on DNA damage leading to its role in carcinogenesis.

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.<sup>14</sup> 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.<sup>15</sup>

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.<sup>16,17</sup> Copper at high concentration is known to cause metastasis of cancer cells.<sup>18</sup> Cobalt(II) complexes are known to produce oxygen radicals which causes heart toxicity.<sup>19,20</sup>

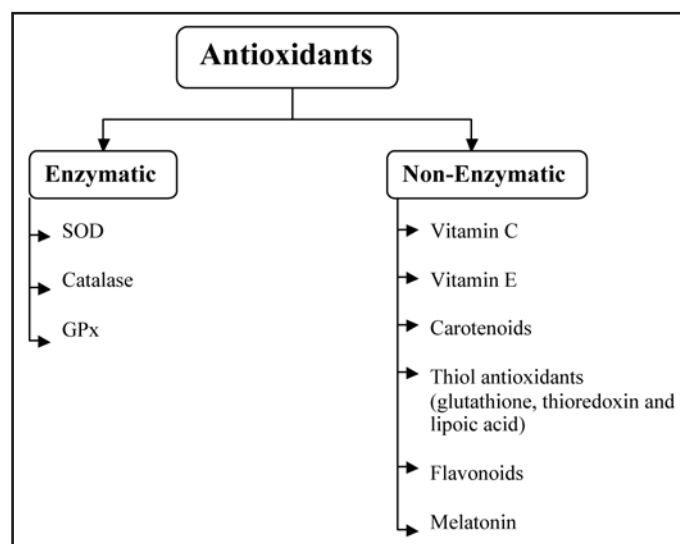
Arsenic is one of the most toxic elements and produces a variety of ROS including superoxide ( $O_2^{\bullet-}$ ), singlet oxygen ( $^1O_2$ ), the peroxy radical ( $ROO^{\bullet}$ ), nitric oxide ( $NO^{\bullet}$ ), hydrogen peroxide ( $H_2O_2$ ), dimethylarsinic peroxy radicals [ $(CH_3)_2AsOO^{\bullet}$ ] and also the dimethylarsinic radical [ $(CH_3)_2As^{\bullet}$ ].<sup>21-24</sup> As(III) enhances the production of heme oxygenase, an indicator of oxidative stress in a variety of human and mammalian cell types<sup>25</sup> and generates free radicals in livers of mice.<sup>26</sup> The production of ROS by various arsenic metabolites was confirmed by animal experiments.<sup>27</sup>

Different mechanisms have been accounted for the toxicity of arsenic. Arsenic (III) compounds bind to sulfhydryl (-SH) groups and can inhibit various enzymes, including glutathione reductase while Arsenic (V) inhibits PDH<sup>28</sup> activity thus causing impaired glucose metabolism.<sup>28,29</sup>

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.<sup>30-32</sup> Depletion of cells' major sulfhydryl reserves e.g.,  $\delta$ -aminolevulinic acid dehydratase (ALAD) seems to be an important indirect mechanism for oxidative stress

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

Alphabetical name	Categories of antioxidants	Examples
Antioxidant C	Carotenoids	$\beta$ -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, Estriol
Antioxidant V	Vitamins	$\alpha$ -tocopherol, Ascorbic acid

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

that is induced by redox-inactive metals.<sup>33,34</sup> 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.<sup>35</sup> 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.<sup>36</sup> Catalase is an efficient decomposer of  $H_2O_2$  and known to be susceptible to lead toxicity.<sup>31</sup> 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.<sup>37</sup> 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.<sup>38</sup> 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 peroxy radical.<sup>47</sup> Metal-catalyzed damage to proteins involves oxidative scission, loss of histidine residues, tyrosine crosslink, the introduction of carbonyl groups, and the formation of protein-centered alkyl,  $R^\bullet$ , alkoxy,  $RO^\bullet$  and alkylperoxy,  $ROO^\bullet$ , radicals.<sup>48</sup>

## 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, nitroxyl 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<sup>49</sup> as well as antioxidant enzymes<sup>50</sup> (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 monodenate, bidenate or multidenate, that is, it may attach or co-ordinate using one or two or more donor atoms. Bidenate ligands form ring structures that include the metal ion and the two-ligand atoms attached to the metal.<sup>51</sup> Their efficacy depends not solely on their affinity for the metal of interest but also on their affinity

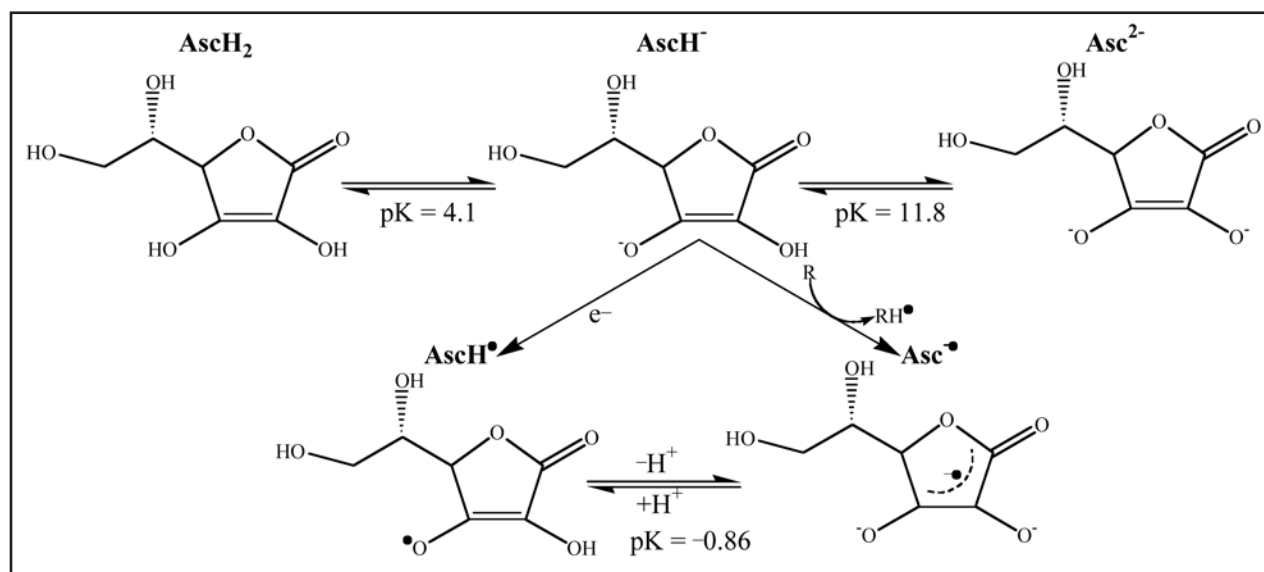


Figure 4. Conversion of ascorbic acid into different reduced forms at various pH indicating possible binding sites and free electrons responsible for their antioxidant and chelating property.

for endogenous metals. 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.

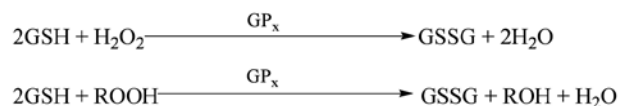
An ideal heavy metal chelator should be able to enter the cell easily, chelate the heavy metal from its complex with metallothionein or other proteins, and increase the excretion of the metal without its redistribution to other organs or tissues.<sup>52</sup> Chelation therapy compromises with various 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  $O_2^{\bullet -}$  to  $O_2$  with remarkably high reaction rates by successive oxidation and reduction of the transition metal ion and to the less-reactive species  $H_2O_2$ .<sup>53</sup>

Catalase is an enzyme present in the cells of plants, animals and aerobic bacteria.<sup>54</sup> 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 anti-oxidative defence mechanisms.  $GP_x$  enzymes in presence of tripeptide glutathione (GSH) add two electrons to reduce peroxides.<sup>54</sup>  $GP_x$  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.



**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  $\alpha$ -tocopherol<sup>55</sup> from  $\alpha$ -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 ( $R^\bullet$ ).<sup>56,57</sup>

Vitamin C scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that inhibits lipid peroxidation.<sup>58,59</sup> 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).<sup>60,61</sup>

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.<sup>62</sup> Vitamin C in plasma increases dose-dependently resistance to-lipid peroxidation, even in the presence of redox-active iron or copper and  $H_2O_2$ .<sup>63</sup>

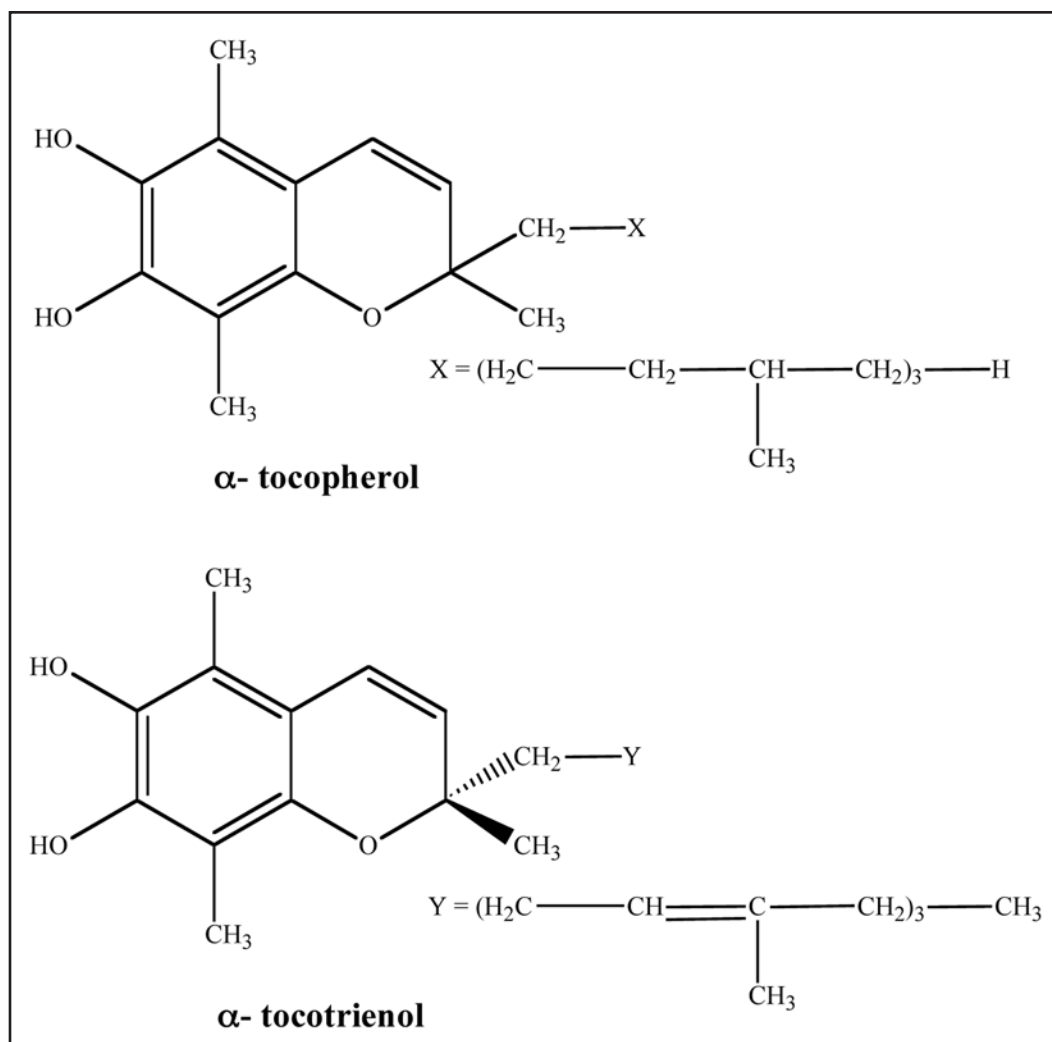


Figure 5. Chemical structure of  $\alpha$ -tocopherol and  $\alpha$ -tocotrienol indicating isoprenoid units.

Other studies indicate the ability of ascorbic acid to regulate factors that may influence gene expression, apoptosis and other cellular functions.<sup>64</sup>

However, studies in humans have shown some mixed results. In a study with 78 male workers, 38 received vitamin C and 38 were given placebo no effect of absorption or excretion of lead was found.<sup>65</sup> An inverse relationship between serum vitamin C and blood lead levels was found in a cross-sectional study which analyzed 4,213 young and 15,365 adult Americans with mean blood lead level of 2.5–3.5 mg/dl.<sup>66</sup> Yet another study among 85 volunteers drinking lead-containing drinking water showed small reductions in lead retention.<sup>67</sup>

A consistent protective effect of Vitamin C has also been found in lung and colorectal cancer.<sup>68</sup>

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 detoxifying agent by forming a poorly ionized but soluble complex.<sup>32</sup> 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).<sup>69</sup> Both structures are similar except the tocotrienol structure has double bonds on the isoprenoid units.

$\alpha$ -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.<sup>70</sup> Its main antioxidant function is protection against lipid peroxidation. During the antioxidant reaction,  $\alpha$ -tocopherol is converted to a  $\alpha$ -tocopherol radical by the donation of labile hydrogen to a lipid or lipid peroxy radical. The  $\alpha$ -tocopherol radical can thus be reduced to the original  $\alpha$ -tocopherol form by



ascorbic acid.<sup>71</sup> 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.<sup>72</sup> Vitamin E has the ability to prevent cell injury by maintaining the sulfhydryl groups of membrane proteins and by quenching free radicals.<sup>73</sup>

Vitamin E has been reported to protect against arsenic toxicity.<sup>74-76</sup> 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.<sup>77</sup> 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  $\text{Fe}^{2+}$  and  $\text{Mg}^{2+}$  from their binding proteins, potentially decreasing the rate of lipid per oxidation.<sup>78</sup>

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.<sup>79</sup> 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.<sup>80,81</sup> 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.<sup>82</sup> Although, the group suggest that vitamin C was better in providing clinical recoveries and Vitamin E was equally efficient in decreasing the lead burden from the tissues.

Studies have also shown that vitamin E reduces the incidence of colorectal cancer by initiating the apoptosis of cancer cells.<sup>83,84</sup>

**Glutathione.** The major thiol antioxidant is the tripeptide, glutathione. Glutathione (GSH) is a multifunctional intracellular non-enzymatic antioxidant which is considered to be the major thiol-disulphide redox buffer of the cell.<sup>85</sup> Generally, the antioxidant capacity of thiol compounds is due to the sulphur atom which can easily accommodate the loss of a single electron.<sup>86</sup> The reaction of glutathione with the radical  $\text{R}^\bullet$  as well as combination with another thiol radical is shown in:

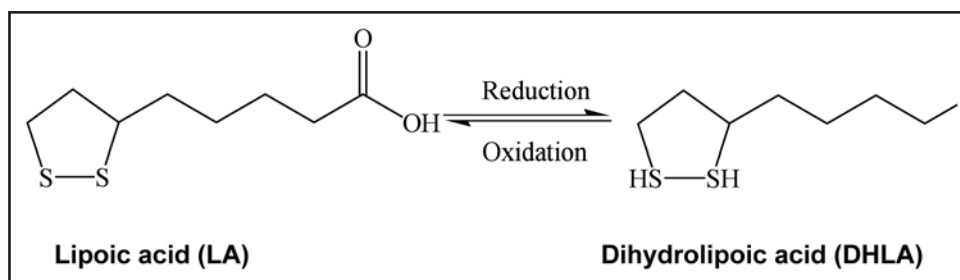


Figure 6. Oxidized and reduced form of lipoic acid showing presence of thiol groups as the possible metal chelating sites.

Oxidized glutathione GSSG is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organisms.<sup>87</sup> Too high a concentration of oxidized glutathione GSSG may damage many enzymes oxidatively. GSSG can react with protein sulfhydryl groups to produce protein-glutathione mixed disulphide:



**$\alpha$ -Lipoic acid.**  $\alpha$ -Lipoic Acid (1,2-dithione-3-pentanoic acid) is a sulfur-containing antioxidant with metal-chelating and anti-glycation capabilities. Unlike many anti-oxidants, which are active only in lipid or aqueous phase, lipoic acid is active in both lipid and aqueous phases.<sup>88</sup> Due to an asymmetric carbon having four different attached groups; lipoic acid exists as a pair of R- and S-. Naturally occurring lipoic acid is the R-form, but synthetic lipoic acid (known as alpha lipoic acid) is a racemic mixture of R-form and S-form in which R-enantiomer is more biologically active than the S-enantiomer.<sup>89</sup>

Lipoic acid is readily digested, absorbed and transported to tissues. A chronic/carcinogenic study in rats reported that racemic lipoic acid was found to be non-carcinogenic and did not show any evidences of target organ toxicity. The No Observed Adverse Effect Level (NOAEL) was considered to be 60 mg/kg bw/day.<sup>90</sup>

LA is readily absorbed from diet and is rapidly converted to Dihydrolipoic acid (DHHLA) by NADH or NADPH in most tissues (Fig. 6). Studies have demonstrated superior anti-oxidant activity of DHHLA as compared to LA. Since DHHLA can neutralize free radicals it is known to regenerate Vitamin C which is even better than GSH<sup>89</sup> and Vitamin E from their oxidized forms.<sup>91,92</sup>

DHHLA possess metal chelating properties which help the body to get rid of accumulated ingested toxins.<sup>93</sup> 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.<sup>92,94</sup> 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%.<sup>95</sup>

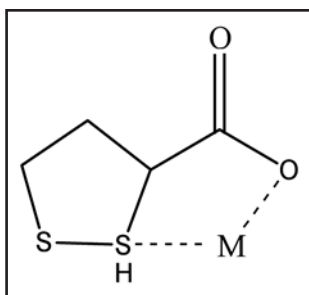


Figure 7. Reduced form of lipoic acid showing metal chelation at thiol groups ( $M = \text{Cu}^{2+}, \text{Zn}^{2+}, \text{Cd}^{2+}, \text{Pb}^{2+}, \text{Hg}^{2+}$  and  $\text{Fe}^{3+}$ ).

LA has been reported to be effective in reducing the amount of  $\text{OH}^\bullet$  generated by Fenton type reactions and also a scavenger of peroxide and  $\text{O}_2^\bullet$ .<sup>96</sup> Sadi et al.<sup>97</sup> 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 peroxy 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.<sup>98</sup> In addition to its antioxidant properties, LA can be neuroprotective in vivo through suppression of glial reactivity.<sup>99</sup> Since chronic reactive gliosis exacerbates diabetic neuropathy, the administration of LA can prevent neuropathy by reducing both oxidative stress and glial hyperactivity.<sup>99</sup> Bhatt et al.<sup>100</sup> 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 free radicals, chelates transition metal ions (e.g., iron and copper), increases cytosolic glutathione and vitamin C levels and prevents toxicities associated with their loss. These diverse actions suggest that LA acts by multiple mechanisms both physiologically and pharmacologically.<sup>101</sup> The relatively good scavenging activity of lipoic acid is due to the strained conformation of the 5-membered ring in the intramolecular disulfide.<sup>102</sup>

Exogenous administration of LA has been found to have therapeutic potential in neurodegenerative disorders also. LA can also cross the blood-brain barrier<sup>103</sup> and is taken up by all areas of the central and peripheral nervous system. Lipid peroxides (LPO) are the presumptive markers of free radical-associated oxidative stress.<sup>104-106</sup> Free radical attack on poly unsaturated fatty acids (PUFA) in the biological system is thought to produce a sequence of reactions, which lead to the formation of both conjugated dienes and lipid hydroperoxides.<sup>107</sup>

Thus the possible mechanisms for the protecting effects of LA against oxidative stress may be as follows: (a) LA can be reduced to dihydrolipoic acid by NADH, (b) DHLA is a strong antioxidant to scavenge excess oxidants, and recycle other antioxidants such as vitamin E, C and glutathione, (c) DHLA chelate metals to prevent free radical generation<sup>92,94,108,109</sup> thus to diminish oxidant attacks on bio-macromolecules, (d) LA is the key co-factor of pyruvate dehydrogenase and  $\alpha$ -ketoglutaric dehydrogenase the enzymes sensitive to oxidative stress, (e) supplementation of sufficient LA helps to fully stimulate activities of enzymes, thereby promoting and ameliorating oxidative phosphorylation and mitochondrial respiration<sup>110</sup> and (f) LA can promote the antioxidant defense by inducing phase two enzymes, such as glutathione synthetase to elevate antioxidant GSH.<sup>111,112</sup>

Lipoic acid (LA) regenerates GSH from GSSG and dihydrolipoic acid (DHLA) recycles ascorbic acid (AA) and dihydroascorbic acid (DHAA). DHLA restores vitamin E (VE) also.

LA satisfies two criteria to be a chelating agent i.e., absorption into the intracellular environment and complexing metals previously bound to other sulfhydryl proteins. Both LA and DHLA can chelate heavy metals, but the R-form is more effective for chelation.<sup>109</sup> LA is most effective in chelating  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Pb}^{2+}$ , but cannot chelate  $\text{Fe}^{3+}$  while DHLA forms complexes with  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Fe}^{3+}$  that are poorly soluble in water. Although DHLA chelates  $\text{Fe}^{3+}$ , it can also reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  which shows its pro-oxidant efficacy. In so far as most iron is tightly bound to ferritin protein, DHLA may have the capacity to remove bound iron from ferritin. DHLA chelation of iron and copper in the brain may reduce free-radical damage contributing to Alzheimer's disease.<sup>113</sup> Small amounts of cadmium ( $\text{Cd}^{2+}$ ) can cause significant lipid peroxidation in the brain, which can be prevented by lipoic acid.<sup>114</sup> Metal chelation by lipoic acid is presented in Figure 7.

LA in unbound form is chemically able to trap circulating heavy metals, thus preventing cellular damage caused by metal toxicity.<sup>114</sup> LA is lipophilic and is able to penetrate cell membranes and reach high intracellular concentrations within 30 seconds of its administration. Oral doses of 10 mg/kg LA in rats reaches peak levels in the cerebral cortex, spinal cord and peripheral nerves within 30 minutes of administration and studies of chronic daily dosing conclude that LA reaches all areas of the central nervous system (CNS) and peripheral nervous system thus decreasing lipid peroxidation in brain and sciatic nerve tissue.<sup>94</sup> Shila et al.<sup>115</sup> reported reduced arsenic concentration in brain tissue of animals supplemented with arsenic and LA. Lipoic acid might also have the capability to interfere with the absorption of arsenic. Beneficial role of LA against lead<sup>116</sup> and GaAs<sup>100</sup> toxicity in terms of lead and arsenic chelation from blood and soft tissues have also been reported.

**N-acetylcysteine.** N-acetyl-L-cysteine (NAC), is a thiol-containing anti-oxidant that has been used to mitigate various conditions of oxidative stress. It reduces liver injury caused by paracetamol over dosage in human<sup>116</sup> and attenuates liver injury and prevents liver and plasma GSH depletion in mice.<sup>117</sup> Its anti-oxidant action is believed to originate from its ability to stimulate GSH synthesis, therefore maintaining intracellular GSH levels

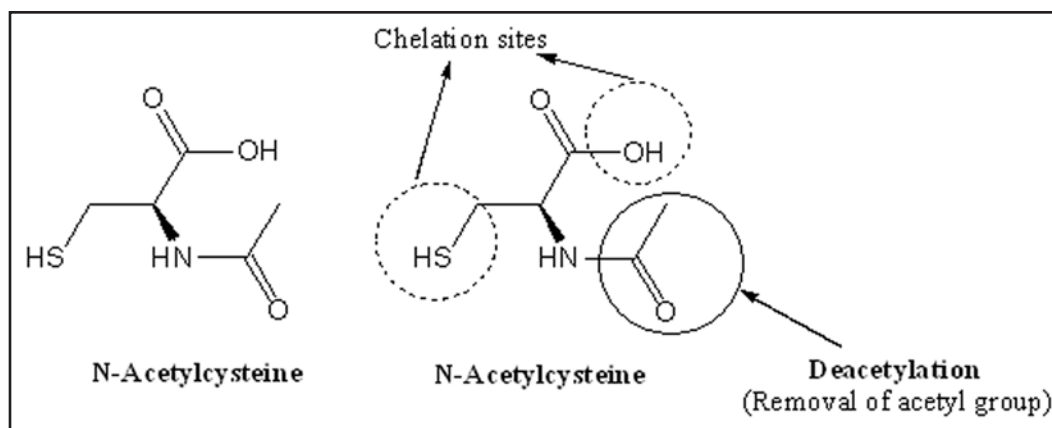


Figure 8. Structure of N-acetyl cysteine (NAC) depicting (1) two chelating sites (thiol and hydroxyl) and (2) deacetylation responsible for its antioxidant potential due to the generation of glutathione.

and scavenging ROS.<sup>118,119</sup> NAC, is rapidly deacetylated to cysteine and thus may increase GSH levels by providing the substrate for the rate limiting step in GSH synthesis.<sup>120</sup> Structure of NAC along with possible chelating sites is presented in Figure 8.

NAC is known to have metal-chelating properties and has been used in several clinical conditions.<sup>121</sup> Thiol groups present in NAC act to reduce free radical and provide chelating site for metals. Thus, NAC has a strong ability to restore the impaired pro-oxidant/antioxidant balance in metal poisoning. NAC can cross the cell membrane therefore provide intracellular effects. Martin et al.<sup>122</sup> reported a better response of acute arsenic symptoms to intravenous NAC than intramuscular 2,3-dimercapto-1-propanol. We recently provided evidence of a novel therapeutic combination to achieve greater effectiveness in chelation treatment against chronic arsenic intoxication in rats.<sup>123</sup> Combined administration of NAC and Succimer post arsenic exposure led to a significant turnover in the variables indicative of oxidative stress and removal of toxic metal (arsenic) from the soft organs.<sup>123</sup> This combination provided a more pronounced reduction in the arsenic induced oxidative stress in liver and brain beside a rapid depletion of soft tissue arsenic burden compared to treatment with DMSA or NAC alone. The major hypothesis behind this new protocol was that, (1) NAC beside a strong antioxidant is a thiol compound and thus, will provide an additional site for metal chelation; and (2) NAC, because of its ability to restore the impaired pro-oxidant: antioxidant balance, will accelerate clinical or biochemical recoveries. Modi et al.<sup>124</sup> proposed protective efficacy of Zn and NAC co-administration against arsenic induced oxidative stress. Santra et al.<sup>125</sup> reported that in arsenic-induced liver cell injury in mice is associated with induction of oxidative stress, the perturbations in the mitochondrial redox state and arsenic-induced apoptosis of hepatocytes and all these changes were reduced in intensity and/or modified by pretreatment with NAC. They showed that on treatment with NAC could replete cellular stores of the tripeptide GSH and are an effective intervention against oxidative stress developed due to arsenic exposure. They also proposed that observed hepatoprotection provided by NAC could be because the liver of

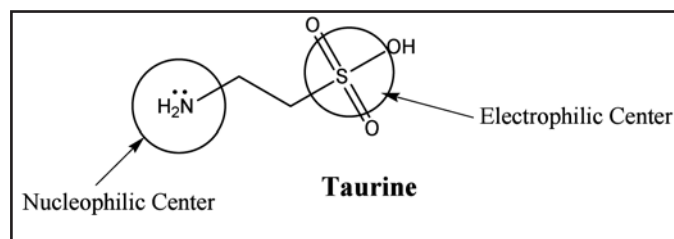


Figure 9. Structure of taurine showing sulfonate group as an electrophilic and amide group as a nucleophilic center responsible for it chelating and antioxidant properties, respectively.

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.<sup>126</sup> One of the first report by Pande et al.<sup>127</sup> 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.<sup>128,129</sup> A recent report suggested that co-administration of NAC along with DMSA in sub-chronically lead exposed rats reduced oxidative stress significantly by lowering the TBARS and GSSG level along with decreased lead burden from soft tissues especially brain.<sup>130</sup>

Beside the antioxidant potential NAC, has also shown to have various other important functions, like the antimutagenic and



anticarcinogenic, in vitro and in vivo. NAC has been demonstrated to inhibit the in vivo DNA adduct formation after administration of aminothiurene or benzopyrene exposure. NAC is also known to protect certain enzymes involved in DNA replication and repair.<sup>131,132</sup>

**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.<sup>133</sup> The zwitterionic nature of taurine gives it high water solubility and low lipophilicity. Consequently compared with carboxylic amino acids, diffusion through lipo-philic 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<sup>134,135</sup> and has been implicated in a wide array of physiological phenomena including long term potentiation in the striatum/hippocampus<sup>136</sup> membrane stabilization<sup>137</sup> calcium homeostasis<sup>138</sup> and protection against glutamate excitotoxicity.<sup>139</sup> It also acts as an antioxidant and protects against toxicity of various heavy metals including arsenic, lead and cadmium.<sup>140-142</sup> Zahorodnyi and Nebesna<sup>143</sup> established that the sulfonate group of taurine is a 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 antioxidant properties.

Low levels of taurine have been associated with retinal degeneration, growth retardation and cardiomyopathy.<sup>144,145</sup> 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.<sup>146,147</sup> 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.<sup>148-150</sup> As a direct antioxidant, taurine is able to quench and detoxify some reactive intermediates such as hypochlorous acid generated by myeloperoxidase<sup>149</sup> nitric oxide<sup>148</sup> and  $H_2O_2$ .<sup>151</sup> On the other hand, as an indirect antioxidant, taurine protect cells via intercalating into the membrane and stabilizing it.<sup>152</sup> The membrane protective effects of taurine are suggested to be related to an action on permeability to ions and water.<sup>150</sup>

In the studies conducted by Gurer and Ercal<sup>80</sup> taurine was shown to have beneficial effects in lead induced oxidative stress in Chinese Hamster Ovary (CHO) cells and rats. An antioxidant mechanisms rather than a chelating activity, seems to underlie this observed effects of taurine against lead-induced oxidative stress. We recently described the dose dependent effect of taurine, either alone or in combination with meso 2,3-dimercaptosuccinic acid (DMSA) in the treatment of sub-chronic lead intoxication in male rats.<sup>153</sup> The results suggested beneficial role of taurine when administered along-with DMSA in providing effective reversal of number of lead sensitive biochemical variables in general, and parameters of oxidative stress in particular, compared to their individual effects. We noted significant effect of taurine when co-administered with DMSA, in depleting blood and brain lead. It is known that highest concentration of taurine is in brain and heart. Perhaps

this in part might explain the significant elimination of lead from the brain tissues. Flora et al.<sup>142</sup> have reported that taurine when administered along with MiADMSA could significantly reduce the arsenic concentration in blood, liver and kidneys. This suggests that the antioxidant capacity of taurine becomes most effective when it is administered along with the thiol chelators or taurine might be facilitating the entry of chelator to the intracellular sites thereby reducing arsenic concentration.

**Carotenoids.** Carotenoids (Car) are pigments that are found in plants and microorganisms. Various studies have indicated that carotenoids may prevent or inhibit certain types of cancer, atherosclerosis, age-related muscular degeneration and other diseases.

The antioxidant activity of carotenoids arises primarily as a consequence of the ability of the conjugated double-bonded structure to delocalize unpaired electrons.<sup>154</sup> This is primarily responsible for the excellent ability of  $\alpha$ -carotene to physically quench singlet oxygen without degradation, and for the chemical reactivity of  $\alpha$ -carotene with free radicals such as the peroxy ( $ROO^\bullet$ ), hydroxyl ( $^\bullet OH$ ) and superoxide radicals ( $O_2^\bullet$ ). At sufficiently high concentrations, carotenoids can protect lipids from peroxidative damage.<sup>155</sup> Carotenoids have antiproliferative effect on various cancer cell lines; lycopene has been shown to inhibit cell cycle progression in breast, lung and prostate cell lines.  $\beta$ -Carotene has been shown to inhibit the expression of anti-apoptotic protein Bcl-2 in cancer cells, reducing thus growth of cancer cells.<sup>156</sup>

**Flavonoids.** Polyphenolic compounds constitute one of the most commonly occurring and ubiquitous groups of plant metabolites and represent an integral part of human.<sup>157-159</sup> 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.<sup>159</sup>

One of the most actively studied properties of flavonoids is their protection against oxidative stress.<sup>158,160</sup> For example, flavonoids are ideal scavengers of peroxy radicals due to their favorable reduction potentials relative to alkyl peroxy radicals and thus, in principle, they are effective inhibitors of lipid peroxidation.<sup>160</sup> 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).

**Quercetin.** Quercetin is one of the most frequently studied bioflavonoid. Flavonoids are polyphenolic compounds possessing 15 carbon atoms, two benzene rings joined by a linear three carbon

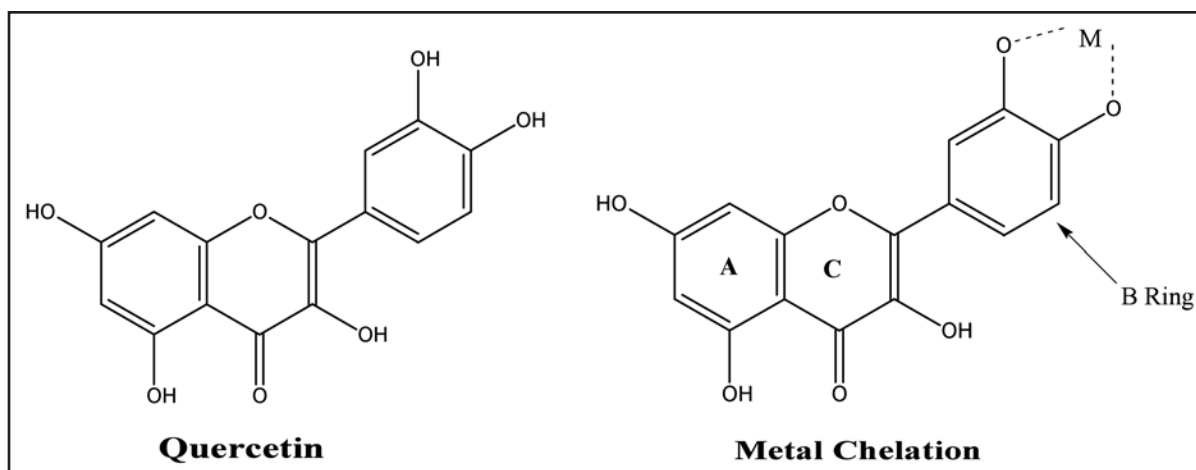
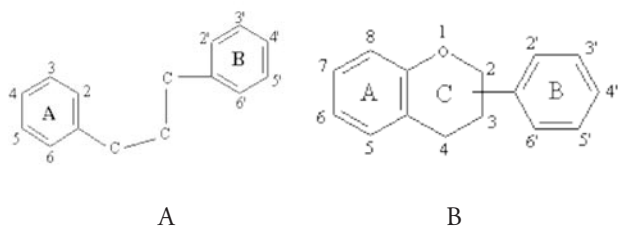


Figure 10. Structure of quercetin showing coordination through the ortho-phenolic groups located on the B ring.

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.<sup>161</sup> It is found in many fruits and vegetables, as well as olive oil, red wine and tea.<sup>162,163</sup> A recent report indicates that blood plasma concentrations may reach in excess of 20  $\mu\text{M}$  quercetin and quercetin metabolites in response to quercetin-rich diets.<sup>164</sup> Quercetin was found to scavenge free radicals and reduce the oxidability and cytotoxic effects of low density lipoproteins.<sup>165,166</sup> High consumption of flavonoid rich food has therefore also been linked to a reduced incidence of cancers at various sites.<sup>167</sup> Quercetin have multiple biological, pharmacological and medicinal properties including anti-inflammatory, anti-allergic, antiviral, antithrombotic, anti-mutagenic, antineoplastic and cytoprotective effects.<sup>168</sup> 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.<sup>169</sup> Quercetin induces growth inhibition and cell death in a variety of cancer cells including glioma cells.<sup>170,171</sup> Quercetin has been reported to have both protective and detrimental effects.<sup>172</sup> 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 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.<sup>173</sup> 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.<sup>174</sup>

Its anti-radical property is directed to scavenge  $\text{OH}\cdot$  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.<sup>175-177</sup> 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 Anaconda,<sup>178</sup> 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 quercetin 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.<sup>179</sup>

Quercetin contains numerous double bonds and hydroxyl groups that can donate electrons through resonance to stabilize the free radicals.<sup>180</sup> 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

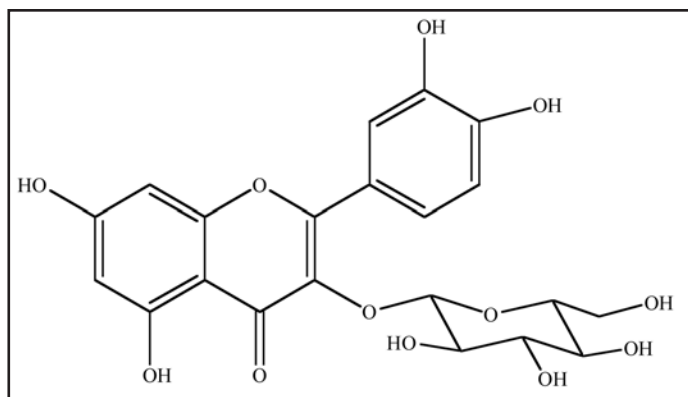


Figure 11. Structure of Quercetin-3-O- $\beta$ -glucoside, a naturally occurring predominant form of Glycosylated Quercetin.

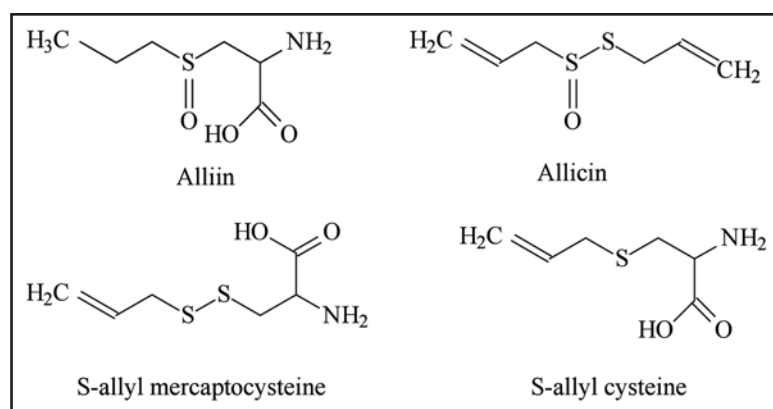


Figure 12. Structures of major constituents of garlic depicting thiol and amino moieties (the possible sites for metal chelation) along with the ketonic, sulphonic and hydroxyl groups (responsible for their antioxidant activity).

processes in cells responsible for degenerative diseases.<sup>181</sup> Quercetin interfere by reacting with the radicals formed in the process of lipid peroxidation.<sup>181-183</sup> Quercetin does not only stop the propagation of lipid peroxidation, but also increases glutathione (GSH) levels.<sup>184</sup> GSH can convert hydrogen peroxide to oxygen and water, preventing the formation of free radicals.<sup>184</sup> Oxidative stress can cause cell death by means of prolonged elevations of intracellular  $\text{Ca}^{2+}$  concentrations.<sup>185</sup> Quercetin can protect cells suffering oxidative stress and thus prevent  $\text{Ca}^{2+}$ -dependent cell death.<sup>185</sup>

The oxidation of low-density lipoproteins (LDL) can result in the formation of atherosclerotic plaques, leading to cardiovascular disease.<sup>181</sup> 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.<sup>186</sup> 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.<sup>187</sup> 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.<sup>181</sup> 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.<sup>187</sup>

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- $\beta$ -glucoside shown in Figure 11.

Glycosylated structures are most common in nature, not the parent compound.<sup>188</sup> 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- $\beta$ -D-glucuronide), are the primary compounds circulating in the blood.<sup>189</sup>

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,<sup>190</sup> found that the quercetin and its conjugate metabolites (quercetin-3-O- $\beta$ -glucuronide and quercetin-3-O- $\beta$ -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.<sup>191,192</sup> Quercetin was found to attenuate oxidative damage induced by arsenic by restoring GSH contents and ROS levels and reducing TBARS levels.<sup>193</sup>

Quercetin has also been reported to increase metallothionein expression and prevent cadmium-induced Nephrotoxicity.<sup>194</sup> 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<sup>195</sup> 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.<sup>100</sup> Mishra et al.<sup>193</sup> 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.<sup>194</sup> 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  $\gamma$ -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.<sup>195</sup> 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 thiosulfonate, L-glutamyl-S-alkyl-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-dithiin, 3-vinyl-4-H-1,2-dithiin, E-ajoene, Z-ajoene, Allyl mercaptan.<sup>196</sup> Structures of some important constituents of garlic are illustrated in Figure 12.

Amagase<sup>197</sup> described formation of thiosulfonates such as Allicin through the enzymatic reaction of sulfur-substituted cysteine sulfoxides, via sulfur-substituted sulfenic acids as a highly reactive intermediate.

According to the recent pharmacological findings, garlic is a preventive rather than therapeutic. The pharmacological effects of garlic have mostly been attributed to its hypoglycemic<sup>198</sup> hypolipidemic<sup>199</sup> anticoagulant, antihypertensive<sup>200</sup> antihepatotoxic, anticancer, immune system modulatory, antiatherosclerotic, antimicrobial, antidote (for heavy metal poisoning) and antioxidant properties.<sup>196,197</sup> 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.<sup>201</sup> Recent studies have demonstrated that garlic exerts its therapeutic effect by increasing nitric oxide (NO) production.<sup>202,203</sup>

It is also found to have free radical scavenging action and inhibits oxidative modification of low-density lipoproteins.<sup>204</sup> The intrinsic antioxidant activity of garlic, aged garlic extracts (AGE) and some garlic constituents have been widely documented in vivo<sup>205</sup> and in vitro.<sup>206</sup> LDL oxidation has been recognized as playing an important role in the initiation and progression of atherosclerosis. Popov and Lewin,<sup>207</sup> observed the antioxidant effect of the aqueous extract from a dehydrated garlic-powder preparation by using photochemiluminescence on the Cu(2+)-initiated oxidation of LDL. AGE and S-allylcysteine (SAC) inhibited both lactate-dehydrogenase release and lipid peroxidation induced by H<sub>2</sub>O<sub>2</sub>. It is also reported that garlic extract shows its antioxidant activity by increases SOD, glutathione peroxidase and catalase activity in vascular cell and culture.<sup>208-211</sup> Furthermore, Geng and Lau,<sup>208</sup> showed that AGE increases intracellular glutathione levels, glutathione disulfide reductase, and SOD activity in bovine pulmonary artery endothelial cells, whereas the level of glutathione disulfide decreased. Geng et al.<sup>212</sup> determined the effects of SAC on NF $\kappa$ B cultivation in human T lymphocytes (Jurkat cells) induced by tumor necrosis factor alpha and H<sub>2</sub>O<sub>2</sub>. SAC consistently inhibited NF $\kappa$ B activation induced by both tumor necrosis factor alpha and H<sub>2</sub>O<sub>2</sub> in nuclear extracts. The results suggest that SAC might act through antioxidant mechanisms to block NF $\kappa$ B activation in Jurkat cells. Allicin also inhibit the growth

of cancer cells of murine and human origin. Allicin induced the formation of apoptotic bodies, nuclear condensation and a typical DNA ladder in cancer cells along with activation of caspases-3, -8 and -9 and cleavage of poly(ADP-ribose) polymerase.<sup>213</sup> Horie et al.<sup>214</sup> demonstrated that AGE prevents the formation of thiobarbituric acid-reactive substances during lipid peroxidation of rat liver microsomes. Among a variety of organosulfur compounds, SAC and S-allylmercaptocysteine (SAMC), major organosulfur compounds found in AGE, showed radical scavenging activity indicating that these compounds may play an important role in the antioxidative activity of AGE. These results suggest that the antioxidant effect of AGE may be due to its modulation of the glutathione redox cycle and SOD activity in vascular endothelial cells.

Sanapati et al.<sup>215</sup> reported the prophylactic efficacy of garlic against lead and found that aqueous extract of garlic have ability to reduce lead burden from soft tissues. Choudhury et al.<sup>216,217</sup> reported co-administration of garlic extract is able to reduce the clastogenic effects of sodium arsenite. Apart from the beneficial role of garlic extract in arsenic induced oxidative stress they suggested the critical role of p53 and heat shock proteins. Recently Flora et al.<sup>218</sup> reported that concomitant administration of garlic extract reduced tissue arsenic burden as well as increased urinary arsenic excretion. This phenomenon could be attributed to the thiosulphur components present in the garlic extract which may act as Lewis acids and interact with Lewis base (arsenic) to form stable components.<sup>219,220</sup> Chowdhury et al.<sup>221</sup> suggested that diverse components of aqueous garlic extracts like allicin also participate in possible chelation of arsenic. These authors suggested that sulphur bearing components of aqueous garlic extract are lipophilic and hence can permeate freely through phospholipids membrane.<sup>221,222</sup>

## 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.<sup>223,224</sup>

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