Chapter 12

Antioxidant Potential of *Asparagus adscendens*

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Additional information is available at the end of the chapter

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1. Introduction

Oxidation reactions give rise to free radicals which are molecules with one or more unpaired electrons in their atomic or molecular orbit (Halliwel and Gutteridge, 1999). Due to the presence of unpaired electrons, free radicals are highly reactive in nature. In biological system, the free radicals namely; reactive oxygen species (ROS) and reactive nitrogen species (RNS) are released during metabolic processes. At lower concentration ROS and RNS play vital roles during mutagenic activity and response to pathogen attack. However, if present in higher concentration it gives rise to oxidative or nitrosative stress (Kovacic and Jacintho, 2001). The accumulation of excess of ROS results in the oxidative degradation of vital biomolecules like lipids, proteins and DNA. This further leads to various diseases like diabetes, cardiovascular diseases and cancer (Dalle & Donne, 2006; Dhalla *et al.*, 2000). The deleterious effect of ROS and RNS is ameliorated majorly through antioxidants. They can be categorized into enzymatic and non-enzymatic in nature. The enzymatic antioxidant includes superoxide dismutase (SOD), glutathione peroxidase and catalase. The non-enzymatic antioxidant includes ascorbic acid (vitamin C), α-tocopherol (vitamin E), carotenoid, thiol antioxidant (glutathione, thioredoxin & lipoic acid) and flavonoids. Antioxidants have therefore been considered as a means to modify and minimize the toxic effect of free radicals. Therefore, they are guardians of human health. Nowadays, mankind is overtly conscious of its health and many have included antioxidants as part of their regular dietary regime. Scientists harbor the popular belief that antioxidants are 'wonder' substances and are working round the clock to discover sources of antioxidants, natural as well as synthetic. Overall there is no convincing evidence that antioxidants in the amounts obtained from fruits and vegetables in the diet have deleterious effect on human health (World Cancer Research Fund, AICR, 1997). The benefits of eating fruits and vegetables may be much greater as compared to the effects imparted by any of the individual antioxidants they contain because the various vitamins, minerals and photochemicals in these whole foods may act synergistically (World Cancer Research Fund, AICR, 1997; Brown *et al.*, 2001). Recent studies have indicated that naturally occurring plants compounds possess

antioxidant properties. A wide variety of plants have been associated with antioxidant effects (Deans *et al.,* 1993; Masaki *et al.,* 1995; Yanishlieva *et al.,* 2006). Several reports describe that the anticancer activity of medicinal plants is due to the presence antioxidants present in them. *Asparagus adcendens* of family Liliaceae, commonly known as safed musli, have been used in Ayurveda and Unani tradition of Indian medicine since long. It has traditionally been used in various ailments including diarrhea, dysentery, leucorrhea and general debility (Kapoor, 2001). It has also been identified as one of the drugs to control the symptoms of AIDS (Trivedi *et al.,* 1993). In the following part of the chapter we throw some light on the different free radicals, their formation, effects and how are they neutralized by plant antioxidant in general and *A. adcendens* in particular.

2. Sources and formation of free radicals

Human body is constantly exposed to the hazards of free radicals. Since free radicals have an unpaired electron in their outer orbit, they are highly reactive and unstable. Their reactivity particularly incurs damage to the vital molecules of human body like lipids, proteins and DNA. There are many biochemical reactions occurring in the human body that prompt the production of ROS. However, they are also triggered by exogenous factors.

To start with, molecular oxygen is released during various metabolic processes which is the preliminary molecule for the production of oxygen free radical. One of the major sources of molecular oxygen is the electron transport chain (ETC) that occurs in the mitochondria and endoplasmic reticulum of the cell. This molecular oxygen is then activated to superoxide radical. During the passing of electron through the various molecules in the ETC, energy is released. However, some electrons leak from the ETC (Salvador *et al.,* 2001; Hanu Kogru 1993). These leaked electrons are responsible for the release of superoxide radical in the mitochondria (Benzi *et al.,* 1992; Brookes *et al.,* 2002).

Other cellular organelles like endoplasmic reticulum consist of cytochrome P 450 in mammalian cells (Butler 1993). Cytochrome P 450 is responsible for the detoxification of toxic compounds carried out by oxidation of foreign/toxic compounds with the aid of enzyme monooxygenase. This process of detoxification is also responsible for the leakage of molecular oxygen to eventually form superoxide radical (Butler 1993). This reaction also occurs in the mitochondria (Thannikal and Fanburg 2000). The enzyme xanthine oxidase, catalyzes the hydroxylation of purine. This reaction of conversion of hypoxanthine to xanthine releases superoxide radical as a by product and the conversion of xanthine to uric acid releases hydrogen peroxide (H_2O_2) as by product (Harrison 2002). Other cellular organelles like microsomes and peroxisomes too release free radical. Microsomes release H_2O_2 which contributes to more than 80% of H_2O_2 produced in the cell (Stohs and Baghchi 1995). The process of oxidation of fatty acid is also a source of H_2O_2 (Fahl *et al.,* 1984). The production of H_2O_2 also takes place in peroxisomes (Valko *et al*., 2004). The H_2O_2 breakdown enzyme catalase, and many metabolic functions that involve consumption of O_2 occur in peroxisomes. Under stress conditions, the superoxide radical is produced in excess which inturn accumulates free iron released from iron containing molecules. This is carried out via

[4Fe-4S] cluster-containing enzyme of dehydratase-lyase family (Liochev and Frodovich 1994). Due to accumulation of excess iron, Fenton reaction occurs, releasing hydroxyl radical (•OH).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH$$

The hydroxyl radical is highly reactive and dangerous. These radicals are also released during the ionized decomposition of water molecule and also photolytic decomposition of alkylhydroperoxidase.

Another free radical found in living organism is the peroxyl radical (ROO•), the simplest form of which exists as hydroperoxy radical (HOO•)and is formed by donating a proton to the superoxide radical ($O_2^{\bullet -}$). This free radical is responsible for the peroxidation of fatty acids.

Like ROS, excessive production of RNS leads to nitrosative stress (Klatt and Lamas 2000). The RNS includes nitric oxide (NO•) which is released in the tissues of organism by enzyme nitric oxide synthase. This enzyme catalyses the conversion of arginine to citrulline and NO• is released as a by product (Ghafourifar and Cadenas 2005). Inspite of being a stable molecule, when produced in higher amount, NO• can bring alteration in the structure and function of proteins.

When superoxide radical and NO• are both produced in large amount, they react together and give rise to a much more reactive free radical called the peroxynitrite anion (ONOO-) which cause DNA fragmentation and lipid peroxidation (Carr 2000).

$$NO\bullet + O2\bullet - \rightarrow ONOO-$$

Other sources of ROS include macrophages and neutrophils when activated during pathogen attack.The macrophages take up oxygen and release free radicals like NO•, $O_2^{\bullet -}$ and H_2O_2 (Conner and Grisham 1996).

Apart from the natural endogenous reaction taking place in the body, the generation of ROS/RNS is also elicited by external/environmental factors like exposure to UV radiations or gamma radiations, carcinogens, heavy metal ions, barbiturates, tobacco smoke and certain pesticides. The various types of ROS, their sources and byproducts are summarized in the table 1.

3. ROS: Damages and diseases

As discussed in the earlier part, it is apparent that due to reasons like environmental pollution, changes in lifestyles, stress and hazards related to work, humans are constantly exposed to the risk of oxidative stress. It has also been well documented that oxidative stress leads to the development of various diseases in humans like diabetes, artherosclerosis, chronic inflammation and ischemia-repurfusion (Behrend *et al.,* 2003; Apel and Hirt 2004; Bergamini *et al.,* 2004). When the ROS species incur damage to vital biomolecules like DNA,

lipids and proteins, irreversible changes occur which mark the beginning of carcinogenesis and ageing.

Type of free radical	Source	Enzymatic/non-enzymatic Antioxidant	Products
Superoxide radical ($O_2^{\bullet -}$)	Electron transport chain, activated phagocytes, Xanthine oxidase	Superoxide dismutase (SOD)	$H_2O + O_2$, H_2O_2
Hydrogen peroxide (H_2O_2)	Product of dismutation of superoxide radical, NADPH oxidase,	Glutathione peroxidase,	H_2O + GSSG
		Catalase,	$H_2O + O_2$
	Xanthine oxidase	peroxidins	H_2O
Hydroxy radical (OH•)	Product of interaction of transition metals like Fe and Cu with $O_2^{\bullet -}$ and H_2O_2		
Nitric oxide (NO)	Nitric oxide synthase	Glutathione, Glutathione reductase	GSNO

Source: Nordberg and Arner 2001

Table 1. The major ROS molecules and their metabolism

Proteins in the form of enzymes catalyse the vital biochemical reactions in the body. Out of 20 amino acids comprising an enzyme, cysteine, methionine and histidine are more susceptible to ROS damage. Thus any enzyme containing these amino acid can undergo inactivation due to presence of ROS, rendering the enzyme inactive, which in turn blocks the vital biological processes.

Lipids that consist of phosphate group are phospho lipids and they are indispensable part of the membranes that surround the cells as well as other cellular structures, such as the nucleus and mitochondria. Lipid peroxidation is one of the major damage encountered by Phospholipds due to ROS. This leads to loss of cellular viability and ageing. If the generation of ROS is triggered by metal ions, then not only DNA but also phospholipids are susceptible to their attack (Siems 1995). This includes damage in the tumor suppressor gene and increased expression of oncogenes resulting in cancer (Wei 1992; Cerutti 1994; Bohr and Dianov 1999). Cancer and diabetes mellitus show a redox imbalance and generation of ROS increases in mitochondria. In such cases, patients have impaired glucose clearance, high glycolytic activity and lactate production.

DNA is the most vital biomolecule and any irreversible change in the DNA base pairing can lead to mutation. ROS is a potential carcinogen and play role in causing mutagenesis, tumor formation and its spread. The damage to DNA caused by ROS involves mispairing mutation

or transversions (G-T) (Higinbotham *et al.,* 1992; Du *et al.,* 1994; Denissenko *et al.,* 1996). Under stress G:C base pair is more susceptible to mutation than A:T base pair. The activation of oncogenes is also a consequence of transversion (Ames 1993). If mutation occurs in p53 tumor suppressor gene, then it gives rise to formation of tumors (Brash *et al.,* 1991; Harris and Hollestein 1993). The p53 gene has an important role in combating cancer and prevention of tumor generation (Hollestein *et al.,* 1991) and also protects the DNA from damage.

Oxidative stress can also enhance the accumulation of compound 8–oxo-dG which is more in the lungs of smokers. This compound is a potential mutagen and can lead to fibrosis and tumor development (Zienolddiny *et al.,* 2000). The presence of increased amount of 8-oxo-dG in the urine of smokers is a reliable biomarker of cancer (Wu *et al.,* 2004). DNA damage caused by oxidative stress due to •OH radical can lead to breast cancer (Jaiyesimi 1992). Hepatitis B and C viruses are activated by oxidative stress caused due to consumption of aflatoxins (Kountouras and Lygidakis 2000; Smela *et al.,* 2000). This activation leads to liver carcinoma (Waris and Siddiqui 2003). Accumulation of 8-oxo-dG is also observed during liver carcinoma (Shwarz *et al.,* 2001; Ichiba *et al.,* 2003). The peroxyl (ROO•) free radical, after its formation undergoes cyclisation to form endoperoxides which act as precursors to malondialdehyde (MDA, the final product of lipid peroxidation) (Fedtke 1990; Mao 1999; Wang *et al.,* 1996). MDA is a potential mutagen in bacterial and mammalian cells. Another byproduct of lipid peroxidation is 4-hydroxy 2-nonenal (HNE) which is also a mild mutagen. These compounds when formed harm the vital molecules to a great extent.

Proteins are also attacked by ROS and in this process mainly residues like cysteine and methionine are oxidized (Stadtman 2004). It leads to the formation of disulphide thiol group. During the oxidation process of protein, the damage can be assessed by the amount of production of carbonyl group which is released (Dalle and Donne 2003). Redox metals are also responsible for carcinogenesis and ageing as they generate free radicals and also bind to thiols (Leonard et al 2004; Santos et al 2005; Valko 2005). Exposure to heavy metals like iron, asbestos and cadmium can lead to cancer (Valko *et al.,* 2001; Stayner *et al.,* 1996; Santos *et al.,* 2005). Cadmium can cause activation of protein kinase which through series of phosphorylation and dephosphorylation increases the expression of downstream genes (Valko 2005). It also plays role in causing pancreatic cancer and renal carcinoma in humans. Chromium causes lung cancer due to high levels of free radicals produced in the mitochondria (Pourahamad and Obrien 2001). Arsenic inhibits the activity of enzymes glutathione reductase by binding to its –SH group. This promotes the DNA damage by UV radiations and also cigarette smoke leading to cancer (Waalkes *et al.,* 2004).

The disruption in signaling cascade due to ROS may cause activation of transcription factors like MAP kinase, activator protein (AP-1) and nuclear factor-κB (NF-ĶB) which is related to cell proliferation and apoptosis (Valko 2006). The activation of AP-1 leads to higher expression of two regulators (c-fos and c-jun) in cell proliferation and can lead to uncontrolled cell division. Due to activation of NF-ĶB, the genes like B-cell lymphoma (bcl-2, bcl-xl), tumor necrosis factor-receptor associated factor (TRAF1, TRAF2), SOD and A20 which can lead to tumor formation in colon, breast, pancreas and other carcinomas (Klaunig and Kamendulis 2004).

ROS and presence of metal ions can also lead to anomalies in the growth factor receptor which causes various cancers (Drevs 2003). Increased expression of growth factor receptor (EGF-epidermal growth factors) has been documented during lung and urinary cancer (Drevs 2003). Amongst all the various categories of cancer and the accumulation of carcinogen, the ROS are observed to play a key role.

4. Antioxidants: Role in prevention and cure

Before the human body succumbs to the deadly biochemistry of free radicals, an array of molecules called "antioxidants" come to its rescue. Antioxidants are the chemicals or enzymes that react with the free radicals and protect the vital biomolecule from the damage by terminating the oxidative chain reaction which is the way to cancer and ageing. Today, it has been well established that by making few changes in the diet by including antioxidants, the occurrence of cancer can be reduced (Khan *et al.*, 2008). There are two categories of antioxidants- enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants include the enzymes that are present in the body to scavenge the ROS/RNS. These include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Mates *et al.*, 1999) (Figure 1). The non-enzymatic antioxidants include- ascorbic acid (vitamin C), α-tocopherol (vitamin E), carotenoid, thiol antioxidants, flavonoids and metallonin (McCall and Frei 1999). Of these, vitamin C, vitamin E and β-carotene are not synthesized in the body and are to be supplied through dietary intake. Table 2 summarizes the dietary sources of various antioxidants.

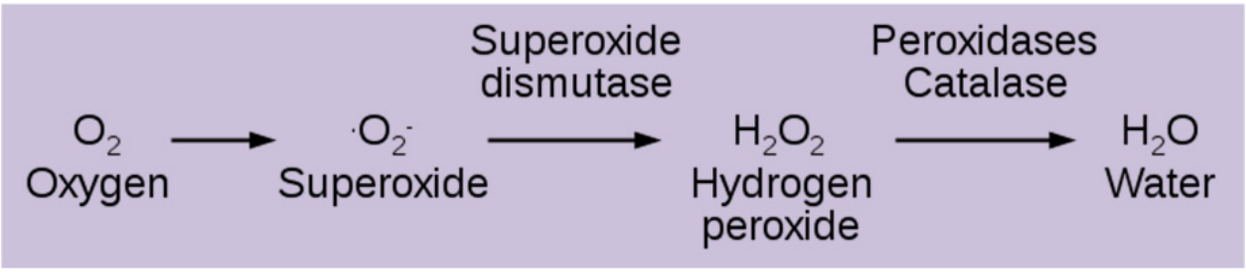

Figure 1. Enzymes involved in detoxification of $O_2^{\bullet -}$ radical.

4.1. Superoxide dismutase (SOD) as antioxidant

SOD catalyses the dismutation of $O_2^{\bullet -}$ to O_2 and H_2O_2 (which is less reactive) (McCord and Fridovich 1969). The SOD exists in different isoforms depending on the type of active metal. In humans, SOD is found in cytosol (cytosolic-SOD which includes Cu and Zn SOD), mitochondria (mitochondrial SOD which includes Mn-SOD) and extra cellular SOD (Landis and Tower 2005).

4.2. Catalase (CAT)

Catalase is present in plants, animals and in aerobic organism and is localized in peroxisomes. It carries out the conversion of H_2O_2 to water and oxygen molecule by the following reaction:

$$H_2O_2 \rightarrow H_2O+O_2$$

It minimizes the concentration of H2O2 at lower level and hence plays role in the prevention of cancer.

Antioxidants	Dietary Sources
Vitamin C	Broccoli, bell peppers, parsley, Brussels sprouts, cauliflower, lemon, strawberries, mustard greens, kiwi fruit, papaya, kale, cabbage, turnip greens, oranges, cantaloupe, summer squash, grapefruit, pineapple, tomatoes, raspberries, spinach, green beans, fennel, cranberries, asparagus, watermelon, and winter squash.
Vitamin E	Almonds, sunflower seeds, hazelnuts, brazil nuts, wheat germ oil, peanuts, peanut butter, breakfast cereals, turnip greens, dandelion greens, spinach, broccoli, blueberries, kiwi fruit and red bell peppers.
Glutathione	Avocados, asparagus, squash, potatoes, okra, cauliflower, oranges, strawberries, cantaloupe, fresh peaches, broccoli, raw tomatoes Grains, eggs, fish, milk, cheese and yogurt
Lipoic Acid	Broccoli, spinach, collard greens, chard, tomatoes, peas, brussels sprouts, Brewer's yeast, carrots, beets, yams and potatoes.
Carotenoids	Carrots, pumpkins, spinach, lettuce, kale, romaine lettuce. beet greens, turnip, cabbage, collard, mustard greens and sweet potatoes.
Flavinoids	Blueberries, cranberries, blackberries, black grapes, raspberries, cherries, red grapes grapefruit, lemons, limes, oranges, apples, pears, plums, peaches, apricots, black, kidney beans, pistachios, cashews, walnuts, pecans, soybean, tomatoes, eggplants, celery, artichoke, snap beans, okra, broccoli, green tea, black tea and red wine.
Selenium	Peanuts, pumpkin seeds, Brazil nuts, almonds, cashews, sunflower seeds, garlic, onions, leeks, broccoli florets, meat, fish dairy, eggs, mushrooms, wheat germ, barley, brown rice, oats, green beans, lima beans, peas, artichokes, okra, leafy salad vegetables, bananas, dates and pomegranates, blackberries, currants and raspberries

Table 2. Types of antioxidants and their dietary sources

4.3. Glutathione Peroxidases (GPX)

These enzymes exist in two forms that differ in number of sub-unit. One is selenium independent glutathione-S-transferase and other is selenium dependent glutathione peroxidase (Mates *et al.,* 1999). The enzymes reduce peroxides to form selenoles (Se-OH).The important substrates of GPX are H_2O_2 and organic peroxide (ROOH).

$$2GSH + H_2O_2 \rightarrow GSSH+2H_2O$$

$$2GSH + ROOH \rightarrow GSSH+ROH+H_2O$$

Glutathione metabolism carried out by GPX is one of the major antioxidant defence mechanism present in the body.

4.4. Ascorbic acid (Vitamin C)

Vitamin C is a very powerful and effective antioxidant which is functional in aqueous environment. The synthesis of vitamin C does not occur in the human, hence it should be taken in the diet. It has two ionisable -OH groups. It is therefore present as diacid ($AscH_2$) and majorly present as $AscH^-$ under normal physiological conditions and in lesser amount it is present as $AscH_2$ and $Asc2^-$. The AscH- combines with free radical to form a tricarbonyl ascorbate free radical (AscH•), which is resonance stabilized and is relatively inert. Thus the production of AscH• marks the end of reaction and protects the organism from the oxidative stress (Kasparov *et al.,* 2005; Cuzzorcrea *et al.,* 2004). Vitamin C also acts a defense against membrane oxidation (Retsky *et al.,* 1999). It plays an important role in inhibiting the reaction between nitrites and amine groups which form N-nitroso compound and provide protection against stomach cancer. It also provides protection against lungs and colorectal cancer (Knekt 1991).

There have been many instances which show the reduction of oxidative stress mediated damage to lipids, proteins and DNA. Recently the role of vitamin C has been seen in gene expression, apoptosis and other vital functions (You *et al.,* 2000). It also regulates the AP-1 signaling pathways, which is responsible for cell proliferation and thus reduces the expression of cancer causing genes.

4.5. α-Tocopherol (Vitamin E)

Amongst the eight related tocopherols and tocotrienols of vitamin E, α-tocopherol is the most active and readily absorbed form. It is fat soluble vitamin and a potential antioxidant. Because of its property of being fat soluble this vitamin can easily get bound to lipid membrane and plays role against lipid peroxidation of the membrane (Pryor 2000). During the redox reaction α-tocopherol is converted to α-tocopherol radical and with the aid of vitamin C, α-tocopherol is regenerated (Kojo 2004). Thus vitamin C and E act in great accordance in water and fat environment, respectively. The supplemental intakes of this powerful antioxidant have been documented to be useful against cancer. Supplemental

doses of vitamin E have been found to incur reduction in the cases of colorectal cancer (White *et al.,* 1997). Vitamin E acts in the prevention of free radical formation also.

4.6. Thiol antioxidants (Glutathione, thioredoxin, lipoic acid)

Amongst the thiol based antioxidants, **glutathione (GSH)** is the most abundantly found intracellular thiol antioxidant. Glutathione (GSH) is the reduced form and glutathione disulphide (GSSH) is the oxidized form. GSH maintains a reducing environment in the cell, hence helps in carrying on the DNA repair and normal expression by protein sulphydryls. GSH acts as a cofactor to many detoxifying enzymes. Glutathione also facilitates the regeneration of vitamin C and E in their active forms and also carries out reduction of tocopherol radical (Valko *et al.,* 2006) (Figure 2). GSH plays role in the redox signaling by both the level of GST and the ratio of GSH/GSSH (Jones *et al.,* 2000). There are many instances when occurrence of cancer has been correlated with disorders of GSH related enzymes and decreased GSH/GSSH ratio (Pastore 2003). GSH also controls many transcription factors like AP-1 and NF-ĸB.

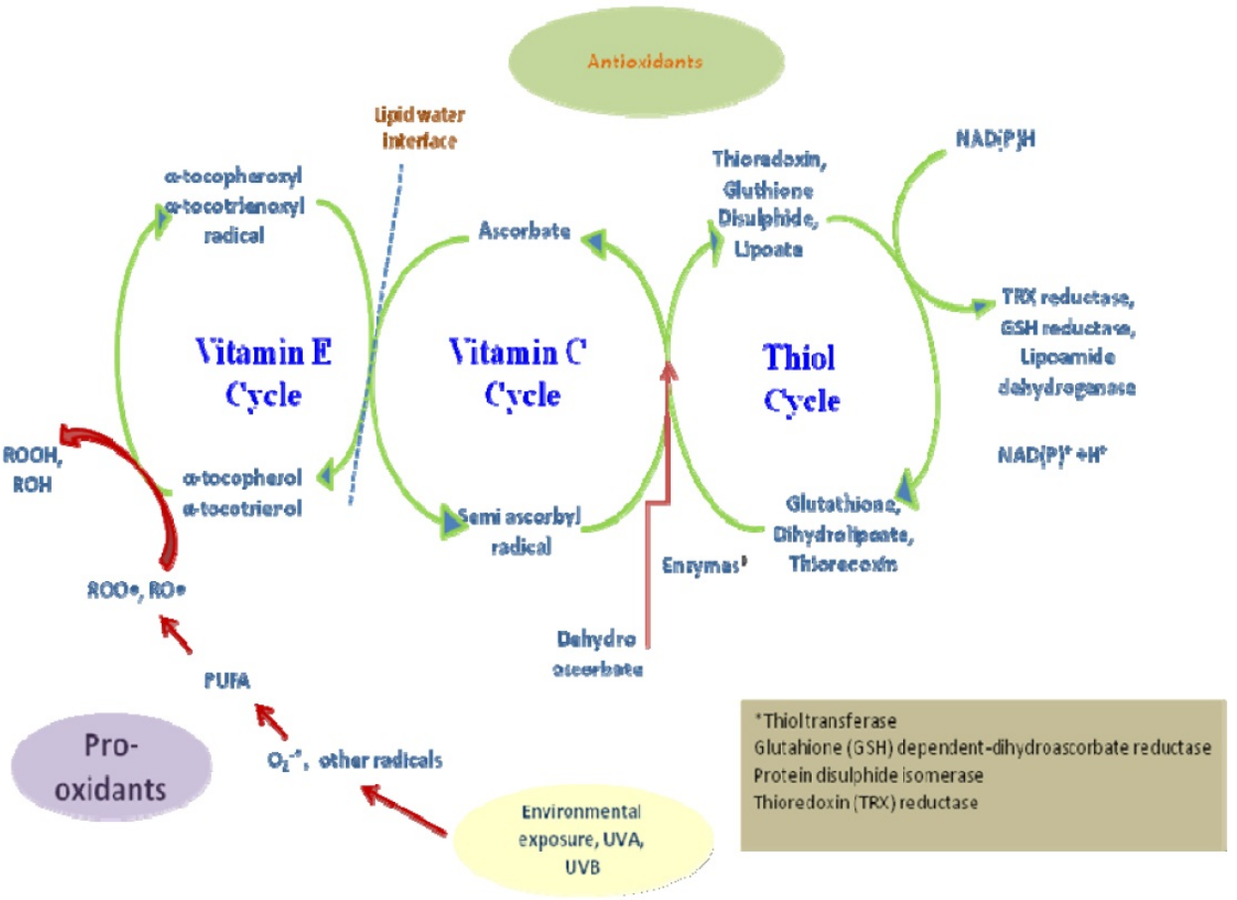

Figure 2. Pathway depicting the interaction between important: Vitamin E, Vitamin C and thiol antioxidants. (Source: Packer *et al.,* 2001)

4.7. Thioredoxin (TRX)

Thioredoxin (TRX) contains 2-SH groups in reduced form which gets converted to disulphide unit in oxidized form. It controls many transcription factors which in turn control cell proliferation. When thioredoxin (TRX) is reduced, it is converted to active state (TR-S) which enters the nucleus. This reaction is catalyzed by thioredoxin reductase. This active and reduced state regulates the activity of transcription factors involved in replication. Thioredoxin also inhibits NF-ĶB and AP-1 transcription factors. The TRX also regulated the hypoxia inducible factor (HIF-1) and also cytochrome P-450. It also regulates the protein by directly binding to them. In the nucleus, TRX regulates the expression of Ref-1 (redox effector factor), which activates p53 transcription factor.

4.8. Lipoic acid or α-lipoic acid (ALA)

Lipoic acid or α-lipoic acid (ALA) or thioctic acid is a disulphide derivative of octanoic acid. It is readily soluble in both aqueous and fat medium due to which it also referred to as "universal antioxidant." It is absorbed from the dietary intake and is stored as dihydro lipoic acid (DHLA) (Smith *et al.,* 2004). Both ALA and DHLA are powerful antioxidant and protect the body by scavenging ROS, regeneration other antioxidants like Vitamin C and E and also chelate metal ions (Cu^{2+} and Fe^{2+}) and prevent the promotion of oxidative chain reaction. There have been many studies in which oral intake of lipoic acid is found to prevent and treat many diseases (Fuchs *et al.,* 1997). The ameliorative effect of lipoic acid has been associated with dreadful diseases like HIV-infection, cardiovascular diseases, neurological disorders, diabetes and also in the diminishing the effect of radiations (Ramakrishnan *et al.,* 1992; Bustamante *et al.,* 1998).

4.9. Carotenoids

Carotenoids are the tetraterpenoid pigment present in chloroplast and chromoplast of plants. They are not synthesized in humans and hence have to be obtained through dietary intake. There are over 600 types of carotenoids divided to xanthophylls (those which contain oxygen) and carotenes (those are made up of only hydrocarbon and do not have oxygen). Carotenoids have the ability to delocalize the unpaired electron through conjugated double bond structure (Mortensen *et al.,* 2001). Due to this β-carotene can efficiently scavenge ROS and also protects the lipid from peroxidative damage. Carotenoids and retinoic acid (a metabolite of vitamin A) regulate many transcription factors and prevent the occurrence of cancer (Niles *et al.,* 2004). The beneficial effect of this antioxidant has been documented during various cancers of breast, lung, prostate, colon and also in leukemia (Karas *et al.,* 2000; Sharoni *et al.,* 2004). In a recent study, the occurrence of oxidative stress in breast cancer survivor with high dietary and plasma carotenoids was much lower than those with low dietary and plasma carotenoid (Thomson *et al.,* 2008). This study indicates important role of carotenoids in oxidative stress mitigation.

4.10. Flavinoids

Flavinoids are the class of plant secondary metabolites. These include 4000 types of flavinoids divided into 13 classes. It is an important class of polyohenolic antioxidants as they play a significant role in curing human diseases (Schroeter *et al.,* 2002). The phenolic antioxidant (Ph-OH) is capable of terminating the oxidation reaction. The following reaction occurs:

$$\text{Ph-OH+ROO}\bullet \rightarrow \text{ROOH+PhO}\bullet$$

Since the PhO• radical so formed is a stable molecule, the propagation of the reaction does not occur. Flavinoids provide a significant result in lessening the rate of various cancers like that of stomach, pancreas, breast and lungs due to their antioxidant properties (Damianaki *et al.,* 2000).

4.11. Selenium

Selenium in a vital micronutrient required by the body (Thomas 2004). It is present in the proteins as seleno-proteins and is an important component of many antioxidant enzymes. The main enzymes are glutathione peroxidases, iodothyronine deiodinases, and thioredoxin reductases. There are many other seleno-proteins with unknown functions. In seleno-proteins, selenium occurs as selenocysteine (Alexander 2007). Selenium plays vital role in protecting the body against the harmful effects of free radicals. Along with vitamin E, selenium promotes formation of antibodies, proteins that act as the body's defense system, helps in carrying out normal biochemical processes and protects the body from risk of cancer. Daily intake of selenium greatly reduce occurrence of some cancers (Harrison *et al.,* 1997). Selenium fights the harmful effects of oxidative stress through involving in processes like, DNA methylation and DNA repair, inflammation, apoptosis, cell proliferation, carcinogen metabolism, hormone production, angiogenesis and immune function (Taylor 2004). It also activates p53 gene, which has a role in cancer prevention. Selenium is present in legumes, cereal grains, soybean, fish, meat and Brazil nuts (Whanger 2002). Recently, it has been found to act as prooxidant and induces apoptosis and is successfully used in anticancer therapy along with other anticancer drugs (Brozmanova 2011).

5. Plants as source of antioxidants

The plant kingdom plays a profound role in the life of humans and animals. Human societies around the world consume a variety of plants and plant products as a food, as masticators items, as spices and condiments, as drink or as herbal medicine. The plants as a source of medicines for different diseases and disorders have been attracting the attention of human mind since ages practically in all societies. Plants are natural chemical factories. The diverse kind of plants flourishing on this planet manufactures a vast variety of natural chemical substances. Dietary plants- such as fruits, vegetables, spices, cereals and edible tubers/roots, which also contain significant levels of bioactive natural compounds, may

provide human health benefits beyond basic nutrition to reduce the risk of many chronic diseases including cancer (Lie, 2003). According to the World Health Organization (WHO), about three quarters of the world's population currently use herbs and other forms of traditional medicines to treat diseases. Recent research have shown that the antioxidants of plant origin with free radical scavenging properties could have great importance as therapeutic agents in several diseases caused due to oxidative stress (Ramchoun *et al.,* 2009). Several other reports describe that the anticancer activity of the plants is due to antioxidants such as vitamins (A, C, E), carotene, enzymes (e.g., superoxide dismutase, catalase and glutathione peroxidase), minerals (e.g., Cu, Mn, Se and Zn), polysaccharides, polyphenols (e.g., ellagic acid, gallic acid and tannins), flavonoids (e.g., quercetin, anthocyanins, catechins, flavones, flavonones and isoflavones), lignins, xanthones, etc (Somkuwar 2003; Kathiresan *et al.,* 2006; Jain *et al.,* 2006). In the developed countries about 25% of all medicinal drugs are said to be based on plants and plant products whereas in the developing as well as underdeveloped countries about 75% of all the medicinal drugs are based on the plants or plant products.

For many decades, ROS generation has been known to cause cellular damage which appears to be a major contributor of many diseases such as aging, arthritis, diabetes, cardiovascular diseases and cancer. Among these, cancer is the leading cause of death all over the world, despite the enormous amount of research and rapid developments seen during the past decade. Recent reports from the International Agency for Cancer Research indicate that in 2008, approximately 12.7 million new cancer cases and 7.6 million cancer deaths occurred and of these, 56% of all new cancer cases and 63% of cancer deaths were in the less developed regions of the world (Ferlay *et al.,* 2010). Projections are that by 2020, the incidence of cancer will increase three-fold, and that there will be a disproportionate rise in cancer cases and deaths from the developing countries that have limited resources to tackle the problem (Are *et al.,* 2010). Cancer is caused by both internal factors (such as inherited mutations, hormones and immune conditions) and environmental/acquired factors (such as tobacco, diet, radiation and infectious organisms). On the basis of current knowledge, it is believed that most of the cancers are not of hereditary origin but because of lifestyle factor such as tobacco, alcohol, diet, obesity, infectious agents, environmental pollutants and radiation. Although the hereditary factors cannot be modified, but a lot improvement can be brought about as far as the lifestyle and environmental factors are concerned. It is believed that consumption of plant products, widely distributed in fruits, vegetables and medicinal plants will be conducive in reducing the incidence of cancer. These substances possess high antioxidant potential. The dietary habits play an important role in prevention of cancer. There have been considerable scientific evidence, epidemiology and experimental, accumulated in these years indicating that a large number of plants, fruits, vegetables and other dietary substances possess efficacy to act as cancer preventive agents (Hickman, 1989, Steinmetz and Potter 1991, Rao *et al.,* 1990). In light of this chemopreventive potential of *A. adcendens* root have been discussed which is mainly because of the antioxidant potential of the plant. Root is used as appetizing, diuretic, aphrodisiac, laxative, astringent useful in dysentery, diarrhea, throat complaints and leprosy (Manandhar, 1980). Root bask is taken with milk for vitality and strength.

6. Chemopreventive potential of *A. adscendens*

Carcinogenesis is a multistep process induced by a variety of carcinogens which ultimately leads to the development of cancer. Many biological and molecular events have been identified which are modulated by different natural agents to inhibit the multiple stages of carcinogenesis. In fact, natural products play a major role in cancer prevention and treatment. It has been also reported that more than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to include apoptosis in various cancer cells of human origin (Rosangkima *et al.,* 2004). Therefore the test diet containing the roots of *A. adscendens,* showed a significant reduction in tumor incidence and tumor multiplicity in skin and forestomach papillomagenesis at all the three doses (2, 4, and 6%) by standard protocol adoption (Singh *et al.,* 2011).

6.1. Effect of *A. adscendens* on carcinogen/Drug metabolism

The parameters that we assessed at the end of the feeding were the inducibility of hepatic enzymes involved in xenobioitc/carcinogen metabolism and maintenance of the cellular antioxidant status. Liver being the major site of xenobiotic/carcinogen metabolism and transformation, the differences observed in this tissue are considered significant. Xenobiotic metabolism plays a crucial role in detoxifying the active carcinogenic dose of a potential carcinogen. Generally, it consists of phase I and phase II metabolizing enzyme systems in which, due to the activity of former, the epoxide can be formed that is an active form of carcinogen known to bind with the DNA, resulting in mutation during cell proliferation. The phase II enzyme system can make it inactive to facilitate their excretion outside the body. As mentioned earlier, reactive oxygen species are intimately linked with the process of carcinogenesis. In this regard, it has been shown that the test diet containing *A.* adscendens modulates both phase I and phase II enzymes including cytochrome p450 reductase, cytochrome b5 reductase, glutathione transferase (GST) and DT-diaphorase (DTD) (Singh *et al.,* 2011).

6.2. Effect of *A. adscendens* on antioxidant status

The plants having chemopreventive potential are known to contain various antioxidants. These antioxidants actively interact with the reactive oxygen species and try to neutralize them. It is well established that free radicals are involved in the initiation and development of cancer. Expectedly, the enzymes involved in the antioxidant function such as catalase, superoxide dismutase were found to be enhanced by test diet containing *A.* adscendens (Singh *et al.,* 2011). Therefore, *A.* adscendens which has ability to scavenge free radicals or interfere with the development process of free radical is expected to inhibit the carcinogenesis. Thus, the balance between antioxidants and oxidants is believed to be a critical concept for maintaining a healthy biological system.

6.3. Effect of *A. adscendens* on peroxidative Damage

In the case of increased antioxidant status, the lowered level of oxidative damage was expected in the group of animals treated with *A.* adscendens diet. To confirm this

possibility, there was a significant reduction in the activity of lactate dehydrogenase, with 4% and 6% test diets of A. adscendens. The lipid peroxidative damage in the hepatic tissue was measured in terms of MDA content. As expected, a significant decrease in the level of peroxidative damage was observed also supported this possibility. The decreased level of peroxidative damage is correlated well in accordance with the induction of antioxidant enzymes above the basal level.

7. Summary

Overproduction of free radicals leads to oxidative stress which is an important contributor to many diseases including cancer. Oxidative stress induces a cellular redox imbalance which may be related to the oncogene stimulation. This harmful effect is counteracted by the antioxidant action of both enzymatic as well as non-enzymatic antioxidants. Thus, antioxidants are important way for body protection against the stress. Since the origin of human civilization medicinal plants have been considered to be imperative source of curing various dreaded diseases and cancer is one among those diseases. There are countless medicinal plants available in nature, which have anticancerous properties and majority of them are still to be explored. Therefore, we evaluated the cancer chemopreventive efficacy of the roots of *A.* adscendens, which have been used in the Indian traditional medicine system since long for the treatment of various ailments. *A.* adscendens in diet was able to inhibit skin and forestomach papillomagenesis induced by DMBA and B(a)P, respectively, in mice. Further the test diet containing roots of *A.* adscendens inhibited phases I and activated II enzymes and antioxidant enzymes. Together these studies suggest that the cancer chemopreventive potential of *A.* adscendens *which could be mediated through drug metabolizing phase I and phase II enzymes; as well as free radical scavenging antioxidant enzymes.* In the future, the identification of all biologically active components could provide mechanistic insight into their preventive and therapeutic potential against various ailments including cancer.

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