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## Review Article

### ANDROGRAPHIS PANICULATA AND ITS BIOACTIVE PHYTOCHEMICAL CONSTITUENTS FOR OXIDATIVE DAMAGE: A SYSTEMIC REVIEW

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#### ABSTRACT

Plants are the source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti oxidant, anti-cancer, antimicrobials etc. *Andrographis paniculata* (Burm. f.) is an herbaceous plant has been widely used for treating sore throat, flu, antioxidants and upper respiratory tract infections. Andrographolide, a major bioactive chemical constituent of the plant, has shown anticancer potential in various investigations. Andrographolide and its derivatives have antioxidants effects in *in-vivo* and *in-vitro* and reduce the free radical damage induced by chemical pollutants. Hence, the present review explains the basic properties of free radicals, free radical mediate damage and its effect on the biomolecules of the *in-vivo* systems. The review also give details on the phytochemical constituents of the medicinal plant *Andrographis paniculata* and its effect on development of antioxidants against free radical induced oxidative damage. However, what is noteworthy about this review is summarizing the effects of bioactive compounds and their role in the development of antioxidants against free radical induced oxidative damage. Therefore, this review is intended to provide evidence reported in relevant literature on qualitative research to assist scientists in isolating and characterizing bioactive compounds with more antioxidants.

**Keywords:** *Andrographis paniculata*, Andrographolide, Free radicals, Oxidative damage.

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#### INTRODUCTION

##### Free Radicals and Oxidative Stress

Oxidative stress has been extensively studied in many model systems and a number of biochemical pathways are involved in determining cell survival or death in response to oxidative stress.<sup>1</sup> In general, free radicals are very short lived, with half-lives in milli, micro or nano seconds. Free radicals have been implicated in the etiology of several human diseases as well as ageing.<sup>2</sup> But it has to be emphasized that ROS and RNS are both produced in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signalling molecules. Most cells can produce superoxide (O<sub>2</sub><sup>•-</sup>),

hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and of redox signalling and with the accumulation of data in various systems, a clearer picture is emerging of the signalling pathways and specific targets affected by ROS/RNS.<sup>3</sup> Recent studies by showed that antibodies, regardless of origin or antigenic specificity, could convert O<sub>2</sub> into H<sub>2</sub>O<sub>2</sub> via a process that they have postulated to involve dihydrogen trioxide (H<sub>2</sub>O<sub>3</sub>).<sup>4</sup> During ischemia-reperfusion, oxidants like O<sub>2</sub><sup>•-</sup>, •OH and H<sub>2</sub>O<sub>2</sub> are produced. This occurs during non-fatal myocardial infarction, surgeries, stroke, transplantation, blockage of arteries under pathological conditions, etc. During ischemia in the heart (in myocyte mitochondria) conversion

of ATP to adenosine causes the generation of  $O_2^{\bullet-}$ , while in the blood vessels (endothelium) the pathway involved is the transition from xanthine to uric acid.<sup>5</sup> Antioxidants are substances that neutralize free radicals or their actions.<sup>6</sup> Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: In every cell superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems.  $\alpha$ -Tocopherol (vitamin E) is an essential nutrient which functions as a chain-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also part of the normal protecting mechanism. Carotenoids, flavonoids and related polyphenols,  $\alpha$ -lipoic acid, glutathione etc are other non enzymatic antioxidants. At different stages antioxidants are capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase (SOD) that catalyses the dismutation of superoxide to  $H_2O_2$  and catalase that breaks it down to water.<sup>7</sup> The relation between free radicals and disease can be explained by the concept of 'oxidative stress'.<sup>8</sup> Other sources of free radicals include redox cycling of xenobiotics, exposure to physicochemical agents like ionizing radiations such as X-rays and  $\gamma$ -rays besides visible light or UV in the presence of oxygen and an endogenous compound or a drug that act as photosensitizer. Most of the damage induced by ionizing radiations in biological systems is indirect and is mediated by products of radiolysis of water including hydrogen radical ( $\bullet H$ ),  $\bullet OH$ , hydrated electron,  $H_2O_2$ , peroxy radical ( $ROO\bullet$ ),  $O_2^{\bullet-}$ ,  $^1O_2$  etc.<sup>9</sup>

## Chemical Properties of Free Radicals Species/ROS

### *Superoxide Ion Radical*

This species possesses different properties depending on the environment and pH. Due to its pKa of 4.8, superoxide can exist in the form of

either  $O_2^{\bullet-}$  or, at low pH, hydroperoxyl ( $HO_2^{\bullet}$ ).<sup>10</sup> The latter can more easily penetrate biological membranes than the charged form. Hydroperoxyl can therefore be considered an important species, although under physiological pH most of the superoxide is in the charged form.

### *Hydroxyl Radical ( $OH\bullet$ )*

The reactivity of hydroxyl radicals is extremely high.<sup>11</sup> The 3 main chemical reactions of hydroxyl radicals include hydrogen abstraction, addition, and electron transfer.<sup>12</sup> In difference to superoxide radicals that are considered moderately stable and have continuous, relatively low reaction rates with biological components, hydroxyl radicals are short-lived species retaining high affinity toward other molecules.  $OH\bullet$  is a potent oxidizing agent that can counter at a high rate with most organic and inorganic molecules in the cell, containing DNA, proteins, lipids, amino acids, sugars, and metals.

### *Hydrogen Peroxide ( $H_2O_2$ )<sup>13</sup>*

The result of the dismutation of superoxide radicals is the production of  $H_2O_2$ . There are some enzymes that can produce  $H_2O_2$  directly or indirectly. Although  $H_2O_2$  molecules are considered reactive oxygen metabolites, they are not radical by definition; they can, however, cause damage to the cell at a relatively low concentration (10 l M). They are freely dissolved in aqueous solution and can easily penetrate biological membranes. Their deleterious chemical effects can be divided into the categories of direct activity, originating from their oxidizing properties, and indirect activity in which they serve as a source for more deleterious species, such as  $OH\bullet$  or  $HClO$ . Direct activities of  $H_2O_2$  include degradation of haem proteins; release of iron; inactivation of enzymes; and oxidation of DNA, lipids, SH groups, and keto acids.

## Free Radicals Induced Herbicide/Pesticide

More than 130 different pesticides are used global for the production of tobacco, with around 25 compounds used on a usual source. They belong to unlike chemical groups, such as organophosphates, carbamates, organo chlorine and heterocyclic pesticides, nitro compounds,

pyrethroids and amides. Many of these compounds can cause moderate to severe respiratory and neurological damage or act as genotoxic and carcinogenic agent. Oxidative stress in biological systems due to the effect of pesticides originates as the result of an imbalance between the generation of oxidizing species and cellular antioxidant defences.<sup>14</sup> Commonly used herbicides such as paraquat, diquat and other related chemicals can be reduced by cellular enzymes and by redox cycling. The herbicides are oxidized to ROS in the presence of molecular oxygen. Smith<sup>15</sup> has reviewed the mechanism of paraquat toxicity in the lung. Paraquat reacts with NADPH and P450 reductase in cells, causing an electron reduction and resulting in the generation of paraquat radicals. This reaction is perpetuated by a cascade of radical reactions and results in the generation of more ROS. The organ-specific toxicity of the lung to these herbicides is considered to be associated with the high oxygen tension and site-specific accumulation of the paraquat in the lung type I and type II cells. This selective accumulation in the epithelial cells is thought to be dependent on a diamine/polyamine transport process located in the cells. The toxicity of these compounds in lung tissue can be diminished by lowering oxygen supply. Recently it was shown that in paraquat-induced lung injury, NO plays a mediator role in pathogenesis.<sup>16</sup> The World Health Organisation reported that roughly 3 million cases of pesticide poisoning occur annually that result in 22,000 deaths worldwide. Many of these chemicals are mutagenic<sup>17</sup> linked to the development of cancers or may lead to development deficits.<sup>18</sup> Rates or amounts of reactive oxygen species (ROS) production can be increased by the presence of a wide range of natural and man-made xenobiotics.<sup>19</sup> The stimulation of free radical production, induction of lipid peroxidation, and disturbance of the total antioxidant capability of the body are mechanisms of toxicity for most pesticides, including organochlorines and pyrethroids.<sup>20</sup> Moreover, ROS alter protein structure or function and Amino acid side-chains can be irreversibly modified into aldehyde or ketone groups

(carbonylation) which can lead to protein aggregation, inactivation or degradation, these changes in protein carbonylation process are a biochemical perturbation resulting from oxidative stress.<sup>21</sup> Induction of oxidative stress is one of the main mechanisms of many pesticides action.<sup>22</sup> The damage to membrane lipids, protein and DNA is the endpoint biomarker of oxidative stress-inducing effects of pesticides.<sup>23</sup> In order to protect the tissue from oxidant injury, antioxidant enzymes are also present in the biological system. Blomhoff<sup>24</sup> reported that when animals are exposed to a dietary oxidative stress, they react with compensatory induction of endogenous antioxidants. The long-term biological hazards associated with the use of organochloride, organophosphate and carbamate pesticides propelled the introduction of a new generation of pesticides with a lesser degree of persistence. In this direction, the use of pyrethroids as insecticidal and antiparasitic formulations has markedly increased as a viable substitute and account for over 30% of insecticides used globally.<sup>25</sup> Pesticide induced oxidative stress as a possible mechanism of toxicity has been a focus of toxicological research.<sup>26</sup> Oxidative stress is a disruption of pro-oxidant and antioxidant balances a result of an increase in reactive oxygen species (ROS) generation, impairment of antioxidant defence systems or an insufficient capacity to repair oxidative damage.<sup>27</sup> Inactivation and removal of ROS depend on the intracellular antioxidative defense systems.<sup>28</sup>

### **Biological Activities of Free Radicals**

Free radicals, known in chemistry since the beginning of the 20th century, were initially used to describe intermediate compounds in organic and inorganic chemistry, and several chemical definitions for them were suggested. Only in 1954 when the pioneering work of Daniel Gilbert and Rebecca Gersham was published<sup>29</sup> were these radicals suggested as important players in biological environments and responsible for deleterious processes in the cell. In 1956, Herman Denham<sup>30</sup> suggested that these species might play a role in physiological events and, particularly, in the aging process.<sup>31</sup> His hypothesis, the free-

radical theory of aging, inspired numerous studies and research efforts and contributed significantly to our knowledge of radicals and, more specifically, oxygen derived radicals and other oxygen derived, non radical reactive species. These metabolites are now considered major players in biochemical reactions, cellular response, and clinical outcome.<sup>32</sup> A crucial advancement in scientific interest in the field of oxygen toxicity and free radicals occurred<sup>33</sup> discovered the role of the protein hemocuprein in the dismutation of superoxide radicals and described the existence of superoxide dismutase (SOD) in almost all aerobic cells.<sup>34</sup> This discovery led to the description of the superoxide theory of oxygen toxicity<sup>35</sup>, which became the focus of much research and debate associated with aging, development, diseases, and cell signalling.<sup>36</sup> Oxygen is required by prokaryotic and eukaryotic cells for energy production, often via the electron transport chain in the mitochondria in the latter. In most cases oxygen is consumed as dioxygen in the form of a diatomic molecule, the configuration that exists in the atmosphere. The source of oxygen has probably been the evolution of the photosynthetic process in blue-green algae.

### **Oxidative-Damage Targets**

The continuous efflux of ROS from endogenous and exogenous sources results in continuous and accumulative oxidative damage to cellular components<sup>37</sup> and alters many cellular functions.<sup>38</sup> Among the biological targets most vulnerable to oxidative damage are proteinaceous enzymes<sup>39</sup>, lipidic membranes and DNA.<sup>40</sup> The chemistry of radicals clarifies the interaction of these species in the locations at which they are being produced. For example, hydroxyl radicals, produced in mitochondrial compartments, are responsible for damage up in the mitochondrion but not the nucleus. Oxygen metabolites that are not particularly reactive, such as HO<sub>2</sub>, may exist in the intracellular situation for longer periods of time and reach locations far from their production site. For example, H<sub>2</sub>O<sub>2</sub> produced in mitochondria may relay away in the cytoplasm or in the nucleolus.

### **Lipids and Lipid Peroxidation**

All cellular membranes are especially vulnerable to oxidation due to their high concentrations of unsaturated fatty acid. The damage to lipids, usually called lipid peroxidation, occurs in 3 stages. The first stage, initiation, involves the attack of a reactive oxygen metabolite capable of abstracting a hydrogen atom from a methylene group in the lipid. The presence of a double bond adjacent the methylene group weakens the bond between the hydrogen and carbon atoms so that it can easily be removed from the molecule. Following hydrogen abstraction, the remaining fatty acid radical retains 1 electron and is stabilized by rearrangement of the molecular structure to form a conjugated diene. When oxygen is in sufficient concentration in the surroundings, the fatty acid radical will react with it to form ROO. during the propagation stage. These radicals themselves are capable of abstracting another hydrogen atom from a neighboring fatty acid molecule, which leads again to the production of fatty acid radicals that undergo the same reactions-rearrangement and interaction with oxygen. The ROO. becomes a lipid hydroperoxide that can further decompose to an aldehyde or form cyclic endoperoxide, isoprotans, and hydrocarbons. The propagation stage allows the reaction to continue. A single initiation can lead to a chain reaction resulting in peroxidation of all the unsaturated lipid in the membrane. An antioxidant that can stop this process is therefore defined as a chain-breaking antioxidant. Fatty acids with no double bonds or with 1 double bond can undergo oxidation but not a chain lipid peroxidation process; for example, oleic acid with 18 carbon atoms and 1 double bond (18:1) cannot undergo the lipid peroxidation process. The last stage, chain termination, occurs following interaction of one ROO. with another radical or antioxidants. Lipids when reacted with free radicals can undergo the highly destructive chain reaction of lipid peroxidation (LP) leading to both direct and indirect effects. During LP a large number of toxic by-products are also formed that can have effects at a site away from the area of generation, acting as 'second messengers'. The

damage caused by LP is highly detrimental to the functioning of the cell.<sup>41</sup> Hydroperoxides are moderately firm molecules, but their corrosion can be moved by high temperatures or by contact to transition metal ions (iron and copper ions). Decay of hydroperoxides generates a complex mixture of secondary lipid peroxidation products such as hydrocarbon gases (e.g. ethane and pentane) and aldehydes (e.g. malondialdehyde (MDA) and 4-hydroxynonenal).

### Carbohydrates

Free radicals such as  $\bullet\text{OH}$  react with carbohydrates by randomly conceptualizing a hydrogen atom from one of the carbon atoms, producing a carbon-centered radical. This leads to chain disruptions in vital molecules like hyaluronic acid. In the synovial fluid surrounding joints, an increase and activation of neutrophils during inflammation produces major amounts of oxy radicals, that is also being related in rheumatoid arthritis.

### Free Radical Destruction to DNA and Cancer

Oxidative damage to DNA is a result of collaboration of DNA with ROS or RNS. Free radicals such as  $\bullet\text{OH}$ ,  $\text{eaq}^-$  and  $\text{H}\bullet$  react with DNA by addition to bases or perceptions of hydrogen atoms from the sugar moiety interaction of  $\bullet\text{OH}$  with purines will generate 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxydeoxyadenosine, form amido pyrimidines and other less characterized purine oxidative products. Several repair pathways repair DNA damage. DNA is a main target of free radical damage. The types of damages made are many and comprise strand breaks (single or double strand breaks), many forms of base damage yielding products such as 8-hydroxyguanosine, thymine glycol or a basic sites, damage to deoxyribose sugar as well as DNA protein cross links. These damages can result in mutations that are heritable change in the DNA that can yield cancer in somatic cells or foetal malformations in the germ cells. The involvement of free radicals with tumor suppressor genes and proto-oncogenes suggest their role in the development of different human cancers.<sup>42</sup> Although DNA is a stable,

well-protected molecule, ROS can interact with it and cause several types of damage: modification of DNA bases, single- and double-DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-protein cross-linkage, and damage to the DNA repair system. Not all ROS can cause damage most is attributable to hydroxyl radicals. For example, following contact of DNA to hydroxyl radicals, like those induced by ionizing irradiation a variety of adducts are formed. The  $\text{OH}\bullet$  can assault guanine at its C-8 position to yield an oxidation product, 8-hydroxydeoxyguanosine (8-OHdG). Other positions could be attacked, and other likely products could be formed. Hydroxyl radicals can also attack other bases like adenine to yield 8 (or 4-, 5-)-hydroxyadenine. Other products are the result of exchanges between pyrimidines and hydroxyl radicals leading to the formation of thymine peroxide, thymine glycols, 5-(hydroxymethyl) uracyl, and other such products.

### Free Radicals, Diabetes and AGEs

Experimental evidences suggest the involvement of free radicals in the onset of diabetes and more importantly in the development of diabetic complications.<sup>43</sup> Scavengers of free radicals are active in preventing experimental diabetes in animal models and in type 1 (IDDM) and type 2 (NIDDM) patients as well as reducing severity of diabetic complications. Insistent hyperglycemia in the diabetic patients leads to generation of oxidative stress due to a) auto-oxidation of glucose; b) non-enzymatic glycosylation and c) polyol pathway. Long-lived structural proteins, collagen and elastin, undergo continual non-enzymatic cross linking during aging and in diabetic individuals.<sup>44</sup>

### Mitochondria, Oxidative Protein Damage and Proteomics

Oxidation of proteins by ROS/RNS can produce a range of stable as well as reactive products such as protein hydro peroxides that can generate additional radicals mainly up on contact with conversion metal ions. Lipofuscin, an aggregate of peroxidized lipids and proteins accumulates in lysosomes of aged cells and brain cells of patients

with Alzheimer's disease.<sup>45</sup> The rapid advance of proteomic methodologies and their application to large scale studies of protein-protein interactions and protein expression profiles suggest that these methods are well suited to provide the molecular details needed to fully understand oxidative injury induced by free radicals.<sup>46</sup> Proteins, also major constituents of membranes, can serve as possible targets for attack by ROS. Among the various ROS, the OH<sup>·</sup>, RO<sup>·</sup>, and nitrogen-reactive radicals predominantly cause protein damage. Hydrogen peroxide itself and superoxide radicals in physiological concentrations wield weak effects on proteins; those containing SH groups, however, can undergo oxidation following interaction with H<sub>2</sub>O<sub>2</sub>. Proteins can undergo direct and indirect damage following interaction with ROS, including peroxidation, damage to specific amino-acid residues, changes in their tertiary structure, degradation, and fragmentation. The consequences of protein damage as a response mechanism to stress are loss of enzymatic activity, distorted cellular functions such as energy production, interference with the creation of membrane potentials, and changes in the type and level of cellular proteins. Protein oxidation products are usually aldehydes, keto compounds, and carbonyls. One of the major adducts that can easily be detected and serve therefore as a marker for protein oxidative damage is 3-nitrotyrosine. This adduct is produced following the interaction between ONOO<sup>-</sup> and other nitrogen reactive radicals with the amino acid tyrosine.

### **Antioxidants and Defence beside Human Disease**

A number of epidemiological studies show that the connection between the levels of recognised antioxidants/phytonutrients present in tissue/blood samples and occurrence of cardiovascular disease, cancer or transience due to these diseases. However, some recent meta analysis show that supplementation with mainly single antioxidants may not be that effective.<sup>47</sup> Oxidative stress induced by oxygen radicals is believed to be a primary factor in various degenerative diseases of the central nervous system as well as in the normal process of

aging.<sup>48</sup> All human cells protect themselves against free radical damage by enzymes such as superoxide dismutase (SOD) and catalase<sup>49</sup> which are disrupted when formation of free radicals exceed its neutralization in the human body. So it is necessary to provide human being with antioxidant for protection from against action of free radicals. Antioxidant is defined as substance that even at low concentration significantly delays or prevents oxidation of easily oxidisable substrate. The main role of antioxidant is to help the body to protect against damage caused by reactive oxygen species.<sup>50</sup> Antioxidant can also decrease mutagenesis and induce carcinogenesis in two ways -by decreasing oxidative DNA damage and by decreasing cell division. Antioxidant agents are natural origin because of their protective effect on humans.<sup>51</sup> Antioxidant action is manifested by a decrease of malondialdehyde (MDA) formation via lipid peroxidation and an increase of hepatic antioxidative enzymes and antioxidants such as glutathione peroxidase (GPX), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and glutathione S transferase (GST).

### **Trace Elements Dependent Antioxidants**

Antioxidants may prevent and/or improve different diseased states.<sup>52</sup> Zinc is an crucial trace element, being a co-factor for about 200 human enzymes, containing the cytoplasmic antioxidant Cu-Zn SOD, isoenzyme of SOD mainly present in cytosol. Selenium is also an essential trace element and a co-factor for glutathione peroxidase. Vitamin E and tocotrienols (such as those from palm oil) are efficient lipid soluble antioxidants that function as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including LDL.<sup>53</sup>

### **Antioxidants Therapeutics in Ayurveda**

Ayurvedic medicines are usually adapted to a specific composition. Indian Ayurvedic and traditional Chinese systems are living 'great traditions' and have important roles in bioprospecting of new medicines from medicinal plants, which are also rich sources of

antioxidants. Recent estimate shows that about 80% of people in emerging countries still depend on traditional medicine-based largely on various species of plants and animals for the crucial healthcare. Ayurveda residues one of the most primordial and yet living traditions accomplished widely in India.

### Indian Medicinal Plants

A part from the dietary sources, Indian medicinal plants also provide antioxidants and these include: (with common/ayurvedic names in brackets) *Aeglemarmelos* (Bengal quince, Bel), *Allium cepa* (Onion), *Allium sativum* (Garlic, Lahsuna), *Aloe vera* (Indian aloe, Ghritkumari), *Amomumsubulatu* (Greater cardamom, Bari elachi), *Andrographis paniculata* (The creat, Kiryat), *Asparagus racemosus* (Shatavari), *Azadirachta indica* (Neem, Nimba), *Bacopamonniera* (Brahmi), *Camellia sinensis* (Green tea), *Cinnamomum verum* (Cinnamon), *Cinnamomumtamala* (Tejpat), *Curcuma longa* (Turmeric, Haridra), *Emblica officinalis* (Indian gooseberry, Amlaki), *Glycyrrhiza glabra* (Yashtimadhu), *Hemidesmus indicus* (Indian Sarasparilla, Anantamul), *Momordica charantia* (Bitter gourd), *Nigella sativa* (Black cumin), *Ocimum sanctum* (Holy basil, Tulsi), *Picrorrhizakurroa*(Katuka), *Plumbagozeylanica* (Chitrak), *Syzigium cumini* (Jamun), *Terminaliabellarica* (Behda), *Tinosporacordifolia* Heart-leaved moonseed, *Guduchi*), *Trigonella foenumgraecum* (Fenugreek), *Withania somnifera* (Winter cherry, Ashwagandha) and *Zingiberofficinalis* (Ginger). There are also a number of ayurvedic formulations containing ingredients from medicinal plants that show antioxidant activities.<sup>54</sup> There are many laboratories from India working on the antioxidant effect of plant compounds, mainly derivative from natural sources that are accomplished of protecting against such damage. Such studies show that compounds with powerful antioxidant activity include carotenoids, curcumin from turmeric, flavonoids, caffeine present in coffee, tea, etc.,

### *Andrographis paniculata* (AP)

*Andrographis paniculata* is an herbaceous plant of family Acanthaceae, genus *Andrographis* native to India and Srilanka. It is usually cultivated in southern Asia. Mainly the leaves and roots were used for medicinal purposes such as traditional siddha and ayurvedic systems of medicine in India and various other countries for several clinical applications. AP also known as “King of Bitters” and has been used for centuries in Asia to treat upper respiratory infection, infections, sore throat, and variety of other chronic and infectious diseases.

### Phytochemical analysis of *Andrographis paniculata*

Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ.<sup>55</sup> There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity.<sup>56</sup> Phenols and phenolic acids, among the simplest bioactive phytochemicals, are known to be toxic for microorganisms. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins.<sup>57</sup> Aqueous extract of *A. paniculata* was screened for various constituents (andrographolide, flavones, lactones) using routine chemical identification methods. Andrographolide is the main constituent and it also active principle of the plant.<sup>58</sup> The phytochemicals of some medicinally important plants like *Andrographis paniculata* of the family Acanthaceae is used to treat infections and some diseases.<sup>59</sup> Phytochemical analysis of *Ad. vasica* show that phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars are present in the leaves. It has also been shown that tannins are biologically active, against *E. coli*, *Staphylococcus aureus*, *Salmonella paratyphi* and *Candida albicans*.<sup>60</sup> The Preliminary phytochemical screening of successive extracts indicated presence of lipids,

flavonoids, saponins, alkaloids, tannins, carbohydrates, terpenoids, and steroids in *F. ramontchi* leaves.<sup>61</sup> The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance: flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities.<sup>62</sup> Alkaloids have been used to treat diseases like malaria, pain killers and managing heart diseases.<sup>63</sup> Generally, glycosides are non volatile and lack fragrance cleaving the glycosidic bond yields the aglycone, which itself may be volatile and fragrant. Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores.<sup>64</sup> Methanolic extract of *A. paniculata* whole plant extract contains different types of phytochemical like flavonoids, tannins, fatty acids, steroids, saponins, anthocyanins, leucoanthocyanins, coumarins, emodins, and phenols which has been shown to possess antibacterial properties against laboratory test organisms like *Staphylococcus aureus*, *Lactobacillus*, *Pseudomonas putida*, *Bacillus subtilis* and *E. coli* of bacteria.<sup>65</sup> It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex compounds.<sup>66</sup> Plant steroids are known to be important for their microbial properties. They are also used in nutrition, herbal medicine and cosmetics.<sup>67</sup> Phenols are a class of low molecular weight secondary metabolites found in most land plants. Phenolic compounds are the largest group of phytochemicals and accounts for most of the antioxidant activity in plants or plant products.<sup>68</sup> At low concentration tannins can inhibit the growth of microorganisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism.<sup>69</sup> Saponin is used as mild detergent and in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins.

### Anti-Oxidant Activity of *Andrographis paniculata* (AP) in Free Radical Induced Damage

In extensive clinical use plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs.<sup>70</sup> Number of plants has been widely used for the treatment of various diseases due to their antioxidant properties for the past few decades. Antioxidants can be defined as compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction and by many other mechanisms and thus prevent disease.<sup>71</sup> It is now well reputable that production of free radicals ( $O_2$ ,  $H_2O_2$  and  $OH$ ) from the incomplete decrease of molecular oxygen during aerobic respiration is closely linked to cellular damage. Regulation of the balance between assembly of reactive oxygen species (ROS) by cellular processes and its exclusion by antioxidant defense system maintains normal physiological processes. Effects of extracts from *A. paniculata* on antioxidant defense system in diverse animal models have been studied from time to time. Intraperitoneal administration of andrographolide, andrographiside and neoandrographolide at a dose of 100 mg/kg BW for 7 days caused a significant elevation in different components of cellular antioxidant defense and reduction in lipid peroxidation. Hydro alcohol extract at same dose showed similar effects.<sup>72</sup> Aqueous extracts also increased the activity of cellular antioxidant enzymes in lymphoma bearing AKR mice.<sup>73</sup> Neoandrographolide scavenged free radicals by donating the allylic hydrogen of the unsaturated lactone ring.<sup>74</sup> Andrographolide exhibited anti-inflammatory effect by preventing oxygen radical production in human neutrophils.<sup>75</sup> Oral dose of aqueous extracts 1g/kg BW showed a significant elevation in antioxidant status in rat urine samples.<sup>76</sup> Ethanolic extracts of leaves, stem and fruits also exhibit anti-oxidant potential.<sup>77</sup> Hydro alcoholic extract also prevents isoproterenol induced increase in lipid peroxidation and increased the activities of antioxidant enzymes viz. super oxide dismutase, catalase, glutathione



peroxidase and the levels of reduced glutathione in hearts of 10 to 12 week old, Wistar male albino rats.<sup>78</sup> Andrographolide also showed a potent free radical scavenging activity *in vitro*.<sup>79</sup> Extracting solvent markedly influenced the antioxidant and TPC activity of *A. paniculata*<sup>80</sup> and the active anti-oxidant compounds were better extracted in methanol for *A. paniculata* numerous ways for centuries. Ethanolic extracts of *A. paniculata* enhanced both antigen specific (observed against humoral as well as Cell-mediated immune response) and nonspecific responses. It was observed that substances other than andrographolide also contributed to the immunostimulant activity of the extract.<sup>81</sup> Treated with *A. paniculata* extract and andrographolide significantly enhanced natural killer cell activity and antibody-dependent cellular cytotoxicity in normal and tumor-bearing animals. Administration in normal as well as tumor-bearing animals an early enhancement of antibody-dependent complement-mediated cytotoxicity was also observed.<sup>82</sup> *A. paniculata* extract or andrographolide resulted in an enhancement of Salmonella-specific antibody response and induction of cell-mediated response against salmonellosis gavage of mice, with an immunized inactivated *S. typhimurium* vaccine.<sup>83</sup> Andrographolide dose dependently suppressed proliferation and cell cycle progression, attenuated DNA-binding activity of NF- $\kappa$ B in endometriotic stromal cells and inhibited COX-2 and TF expression. It also significantly reduced lesion size in a dose-dependent manner and significantly increased response latency. Andrographolide treatment also significantly reduced immunoreactivity of COX-2, phosphorylated p50 and p65, and NGF in ectopic endometrium.<sup>84</sup> It was also useful in inflammation related immune dysregulatory diseases like allergic asthma, rheumatoid arthritis and neurodegenerative disease via inhibition of the NF- $\kappa$ B signalling pathway.<sup>85</sup> It also interfered with T cell activation and reduced experimental autoimmune encephalomyelitis in mice.<sup>86</sup> Pre-treatment with *A. paniculata* reversed cyclophosphamide (CYP) potentiation of the

delayed-type hypersensitivity (DTH) reaction. Furthermore, treatment with the extract elevated the depressed hemagglutination antibody (HA) titer and increased the number of plaque-forming cells (PFCs) in the spleen cells of mice that had been treated with CYP and challenged with sheep red blood cells (SRBC). It was also found that treatment with the extract stimulated phagocytosis in mice. A significant increase in total WBC count and relative weight of spleen and thymus also occurred in mice.<sup>87</sup> Pre treatment and post-treatment with *A. paniculata* exhibited a significant protective effect against lipo polysaccharide (LPS)-induced neurotoxicity in mixed neuron-glia cultures, *A. paniculata* also significantly attenuated LPS-induced microglial activation and production of reactive oxygen species, tumor necrosis factor, nitric oxide, and prostaglandin E2. Furthermore, *A. paniculata* dose-dependently attenuated LPS induced inducible nitric-oxide synthase and cyclooxygenase-2 protein expression in BV-2 microglia, as determined by Western blot.<sup>88</sup> All these findings suggest that *A. paniculata* could be a useful “natural complement” for immune boosting. Verma and Vinayak compared the antioxidant effects of the aqueous extract on liver defense systems in lymphoma-bearing AKR mice. The aqueous extract significantly increased the activities of catalase, superoxide dismutase, and glutathione-S-transferase enzymes and reduced lactate dehydrogenase activity.<sup>89</sup> Anti-oxidative activity of *A. paniculata* contributes to its anti-inflammatory, anti-cancer, anti-hepatotoxic, anti-atherosclerotic and anti-diabetic activities.<sup>90</sup> An *in vivo* study demonstrated that the *A. paniculata* 80% ethanol extract enhanced murine hepatic antioxidative enzymes and antioxidants such as GPX, GR, CAT and SOD but reduced lipid peroxidation.<sup>91</sup> The *A. paniculata* methanol extract significantly lowered MDA levels and raised the total antioxidant status in urine samples 24 hours after oral administration.<sup>92</sup> The *A. paniculata* methanol extract preserved CAT and SOD activities in erythrocytes after CCl<sub>4</sub> administration.<sup>93</sup> Oral administration of the *A. paniculata* aqueous

extract significantly enhanced CAT, SOD and GST activities in the liver of lymphoma bearing mice.<sup>94</sup> Moreover, the *A. paniculata* aqueous extract exhibited more antioxidant action than its ethanol extract in terms of free radical scavenging, xanthine oxidase inhibition and anti-lipid peroxidation.<sup>95</sup> In recent years, there is a tremendous interest in the possible role of nutrition in prevention of disease. In this context, antioxidants especially derived from natural sources such as medicinal plants and herbal drugs derived from them require special attention. Antioxidants neutralize the toxic and 'volatile' free radicals. Antioxidants have many potential applications, especially in relation to human health, both in terms of prevention of disease and therapy.<sup>96</sup> In biological systems oxygen gives rise to a large number of free radicals and other reactive species collectively known as 'reactive oxygen species' (ROS). Another group of reactive species are termed as 'reactive nitrogen species' (RNS).<sup>97</sup> In a normal healthy human, the generation of ROS and RNS are effectively kept in check by the various levels of antioxidant defence. However, when the humans get exposed to adverse physiochemical, environmental or pathological agents this delicately maintained balance is shifted in favour of pro-oxidants resulting in oxidative stress.<sup>98</sup> Cellular damage induced by oxidative stress has been implicated in the etiology of a large number (>100) of human diseases as well as the process of ageing. Various antioxidants may prevent and/or improve diseased states.<sup>99</sup> These include the intracellular antioxidant enzymes and the dietary or oral supplements in the form of vitamin C, vitamin E,  $\beta$ -carotene, zinc and selenium.<sup>100</sup> Antioxidants also can act at different levels of protection such as prevention, interception and repair. The ethanolic extract prevented the toxicity of kidney from atrazine exposure. The antioxidant activity of AP suggests it as potential antioxidant against atrazine induced oxidative stress.<sup>101</sup>

#### **Hepatoprotective Activity of *Andrographis paniculata* (AP)**

Liver diseases remain one of the severe health problems. In the lack of consistent liver defensive

drugs in allopathic medical practices, herbs play a role in the managing of various liver disorders. A number of plants have shown hepatoprotective property.<sup>102</sup> *Andrographis paniculata* (Kalmegh) is used extensively in the Indian traditional system of medicine as a hepatoprotective and hepatostimulative agent. The aqueous extract of the leaves of this plant has traditionally been used for treatment of various liver disorders and jaundice. To study the influence of the aqueous extract of *Andrographis paniculata* (AP) on severe liver damage leading up to a carcinogenic condition. Hepatic dysfunction due to ingestion or inhalation of hepatotoxin is increasing worldwide.<sup>103</sup> *A. paniculata* has been extensively studied for its efficacy against hepatic toxicity. Kalmegh extract has been shown to completely revert the ethyl alcohol induced damage in rat liver at a dose of 0.5mg/kg.<sup>104</sup> The extract characteristically inhibited hepatic microsomal aniline hydroxylase, N-demethylase and o-demethylase.<sup>105</sup> Single or repeated oral administration of the plant extract 500 mg/kg showed significant change in NADPH induced hepatic microsomal lipid peroxidation. It prevented liver from CCl<sub>4</sub> induced toxic injury.<sup>106</sup> The extract also reduced carbon tetrachloride (CCl<sub>4</sub>) induced elevation in microsomal lipid peroxidation.<sup>107</sup> *A. paniculata* extract inhibited ethoxyresorufin-O-deethylation activity in rat and human liver microsomes, with apparent K<sub>i</sub> values of 8.85 and 24.46  $\mu$ M, respectively. In each case, the mode of inhibition was non-competitive. *A. paniculata* is extensively used as a hepatostimulant and hepatoprotective agent in Indian systems of medicine.<sup>108</sup> *A. paniculata* is also an ingredient in several polyherbal preparations used as hepatoprotectants in India,<sup>109</sup> one of which has been reported as efficacious in chronic hepatitis B virus infection.<sup>110</sup> Very few studies on the effects of crude extracts of *A. paniculata* on liver function are available. Most studies for hepatic effects have been conducted on either andrographolide or other purportedly active principles. Shukla *et al.* reported significant choleric effects of andrographolide in conscious rats and anesthetized guinea pigs. The protection

of andrographolide against acetaminophen-induced reduction in volume and contents of bile was better than that produced by silymarin.<sup>111</sup> Multiple-dose pretreatment with arabinogalactan proteins and andrographolide was protective against ethanol induced hepatotoxicity in mice and was deemed comparable to the efficacy of silymarin.<sup>112</sup> Choudhury and Poddar reported that oral pre- and post-treatment of adult rats with an extract of *A. paniculata* was protective against ethanol-induced increase in serum transaminases. Administration of the extract to normal adult rats in single and multiple doses for seven and 15 consecutive days did not significantly affect serum transaminases.<sup>113</sup> A comparative study on the effect of leaf extract or andrographolide on carbon tetrachloride (CCl<sub>4</sub>)-induced hepatic microsomal lipid peroxidation revealed a protective effect of a single oral dose of the extract and of andrographolide. However, high concentration CCl<sub>4</sub>-induced microsomal lipid peroxidation *in vitro* was completely protected by the extract but not by andrographolide, indicating that the hepatoprotective effect is not solely due to the presence of andrographolide.<sup>114</sup> Hepatoprotective effects of the crude alcohol extract of leaves against CCl<sub>4</sub>-induced liver damage have also been reported by Rana and Avadhoot.<sup>115</sup> The efficacy of *Andrographis paniculata* (AP) extract was studied on atrazine induced hepatic damage in rats. AP treatment ameliorated the effects of atrazine suggesting it as potential antioxidant against atrazine induced oxidative stress.<sup>116</sup> Handa and Sharma compared andrographolide, methanol extract of the whole plant containing equivalent amounts of andrographolide, and an andrographolide-free methanol extract against CCl<sub>4</sub>- induced liver damage in rats. The CCl<sub>4</sub>-induced increases in serum transaminases, serum alkaline phosphatase, serum bilirubin, and hepatic triglycerides were inhibited by 48.6-, 32- and 15 percent, for andrographolide, methanol extract, and andrographolide-free methanol extract, respectively. Since all three treatments resulted in improvement in liver histology,<sup>117</sup> a hepatoprotective role of *A. paniculata*

constituents other than andrographolide is suggested and corroborates the observation made by Choudhury and Poddar.<sup>118</sup> The CCl<sub>4</sub>-induced increase in pentobarbitone induced sleep time in mice is also completely normalized by andrographolide. The effects of intraperitoneal (i.p.) pretreatment for three consecutive days with andrographolide on CCl<sub>4</sub>- or tert-butyl hydroperoxide induced hepatotoxicity in mice were compared with two other diterpenes-andrographiside and neoandrographolide. Both compounds showed a greater protective effect than andrographolide. The protection by andrographiside and neoandrographolide was comparable to silymarin and neoandrographolide normalized glutathione levels.<sup>119</sup>

### Novel Approaches For Antioxidants Development

Antioxidant-based drugs/formulations for prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease (AD), Parkinson's disease, cancer, etc. appeared over the ancient times three decades. Free radical theory has radically moved interest in the purpose of dietary antioxidants in preventing many human diseases, including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration and diabetes. Dietary antioxidants may have capable therapeutic prospective in delaying the onset as well as in preventing the ageing population with AD and its related difficulties. Two neuroprotective clinical trials are accessible with antioxidants: Deprenyl and tocopherol antioxidant therapy of Parkinson's study. By fusing ancient insight and modern science, India can create world-class products. Therefore, it has embarked on a fast track programme to discover new drugs by construction on traditional medicines and viewing the unlike plants and microbial sources of the country. In terms of its size, diversity and right to use to capacity and resources this programme is not only the world's prime project of its kind, but is also sole (Jayaraman, 2003).<sup>120</sup> Ayurveda, antioxidants and therapeutics employing a unique holistic approach, Ayurvedic medicines are usually adapted to an individual constitution. Ayurvedic

Indian and traditional Chinese systems are living 'great traditions' and have important roles in bioprospecting of new medicines from medicinal plants, which are also rich sources of antioxidants. Current estimate indicates that about 80% of people in developing countries still rely on traditional medicine-based largely on various species of plants and animals for their primary healthcare. Ayurveda relics one of the most ancient and so far living traditions practiced widely in India.

## CONCLUSION

Free radicals have been implicated in the etiology of large number of major diseases. They can adversely change many crucial biological molecules leading to loss of form and function. Such undesirable changes in the body can lead to diseased circumstances. Antioxidants can protect against the damage induced by free radicals acting at various levels. Recent research centers on various strategies to protect crucial tissues and organs against oxidative damage induced by free radicals. The traditional Indian medicinal plants are rich sources of natural antioxidants. The bioactive compounds from *A. paniculata* have potential application in the development of antioxidants, anti inflammatory and other disorders. Coordinated research involving biomedical scientists and physicians can make significant difference to human health in the coming decades. Research on free radicals and antioxidants involving these is one such effort in the right direction of healthy society.

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