

FREE RADICALS IN AYURVEDA

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Abstract: There has been considerable interest in free radicals and their control by extracts of medicinal herbs. This article reviews the advances made in this frontier area of research.

INTRODUCTION

As we know, the genetic control, more specially the transcriptional and the translational regulations control the whole physiology of a living cell. Similarly the three body humours *Vata*, *Pitta* and *Kapha* have the key of life, as per ayurvedic concept. All the diseases are the outcome of the imbalance of these humours or the disturbed genetic expression of different macromolecules. Similarly the treatment part is also holistic in ayurveda, which means, by treating the basic body hours, all the diseases could be cured. Therefore, the diseases and their treatment part is also divided into 3 basic groups. This is the baseline, which could be developed to understand the scientific basis of ayurvedic philosophy.

The possible correlation between these ancient concepts and the established modern theories of some diseases, with special reference to the free radical mediated diseases and vata disorders will be discussed here.

Free Radical Reactions and Herbal Antioxidants

With the development of earth planet, living system came into existence as the anaerobic organism but slowly the captured transition

metal from the environment and used sunlight as the source of energy. This process, later named as photosynthesis, produced oxygen which finally introduced the existence of aerobic organisms. These living entities acquired oxygen and iron as the essential components for their sustainability.

Although these two elements are essential for the existence of the existence of a living aerobic organism but they have several side effects also. So nature has simultaneously developed the defence mechanism against the toxicity, created by these essential elements. In other words the use of Fe and O_2 is a double edged weapon.

During the process of metabolism the unwanted molecules are produced as the by product of the aerobic processes within the mitochondria which are now named as free radicals, They are defined as molecules having unpaired electrons in the outer orbit and have ability to participate in the 1 electron transfer reactions. They are highly reactive in nature, thus also known as reactive in nature, thus also known as reactive oxygen species.

If we consider the electronic configuration of oxygen, it is thermodynamically inert but

from electropotential point of view, it is highly active. In the native state, it has two unpaired electrons with parallel spin in its outermost orbit, which restricts its reactivity.

Mitochondria is the power house of cell, NADPH produced in TCA cycle passes through the electron transport chain and its hydrogen-electron gradually reduces the oxygen which is the ultimate electron acceptor of this reaction leading to the formation of water. In this redox reaction there is addition of 4 electrons to a molecule of oxygen for its complete reduction, but 1-5% of oxygen leaks out of this system and produces superoxides. This ultimately enters to a chain reaction of free radical generation and thereby lipid peroxidation.

Free Radicals:

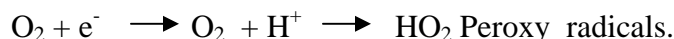
Free radicals are reactive molecules and atoms, having unpaired electrons. They have short life and are formed by (1) homolytic cleavage of covalent bonds where each fragment retains one electron or (2) by loss of single electron or (3) by addition of single electron. For attaining the stability,

these free radicals abstract an electron from a stable molecule, converting it to a new free radical. This leads to a chain reaction which can be divided into three steps: (a) initiation (b) propagation (c) termination. It can be interrupted by using chain breaking antioxidants or it can be terminated by destroying free radicals through enzymes or scavengers.

Generation of free radicals

The generation of free radicals may be accidental or deliberate and the major source should be the leakage from Electron transport chain. FR generation is a chain process. The reaction between H_2O_2 , Fe^{3+} and O_2 produces OH radicals and several other radicals. In addition to this reaction, superoxides are also converted to singlet oxygen where one of the unpaired electron is moved to high energy orbital and changes its spin direction, it gets paired and also reacts with the double bonds of all the macromolecules present in the cell, the output of the chain reaction is different types of free radicals and proxy radicals as shown here.

At this moment it would be better to discuss the different species of reactive oxygen species.

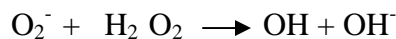


H_2O_2 is produced by $2e^-$ reduction of oxygen.

When two superoxides dismutate then H_2O_2 is formed



H_2O_2 is not a free radical but it produces OH radicals which are highly reactive - 2 ways.



Reducing agents continue the cycle of conversion of $Fe^{3+} \longrightarrow Fe^{2+}$ like ascorbate.

$\text{OH}_2 \rightarrow$ By protonation of superoxides. It is less polar than O_2^- , so easily traces the biological membrane. It is more reactive than O_2^- and can directly attack on the fatty acids.

Sources of ROS

(1) Exogenous interaction- UV, X-rays, chemicals

(2) Endogenous mitochondrial leakage

Sites of free radical generation within the cell are mitochondria, lysosomes, peroxisomes, nuclear membrane, endoplasmic reticulum and plasma membranes.

Free radicals mediated damages can attack on every double bond - Brings deformity to enzymes, nucleic acids through base hydroxylation, cross linking and strand cession.

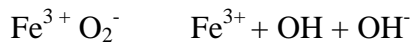
Collagen and hyaluronic acid are also vulnerable o FR attack. Role of iron 1:1 – 1:7 Fe^{2+} : Fe^{3+} is essential for LPO.

Pure lipid peroxides are fairly stable, but when they react with Fe Alkoxy radicals RO_2 peroxy radicals RO_2 are produced.

Haber-Weiss reaction:



Fenton reaction:



Free radicals are also produced during respiratory burst in neutrophils and macrophages. Is generation is enhanced b certain exogenous sources including toxic foreign bodies, radiations, car smoke, hydrophobic chemicals, pathogens, carcinogenic substances, tobacco smoke, pesticides, hyperoxic environment, anaesthetics and organic solvents.

Oxygen containing free radicals are the most critical species of radicals. When oxygen is exposed to high energy such as radiation, or undergoes partial reduction in the electron transport chain, the molecular oxygen is converted to singlet oxygen or superoxides. Here, the two electrons have parallel spin which make it unstable and reactive, further, true oxygen species produce superoxide radicals, second way of reduction of molecular oxygen radicals, second way of reduction of molecular oxygen is by transfer of electrons leading to superoxides which produce hydrogen peroxide after dismutation.

These products react to form water and hydroxyl radicals. Superoxides and transition metals may also react with H_2O_2 to generate hydroxyl radicals known as haberweiss and fenton's reaction respectively. Ferrous (Fe^{++}) and cuprous (Cu^+) ions are much more reactive than their oxidized counterparts.

Besides oxygen radicals, there are other free radicals specially carbon centered radicals @ They are formed by the attack of OH radicals on lipids, nucleic acids carbohydrates and proteins, free radicals are also generated by auto oxidation of small molecules such as thiols and catecholamines and by the activity of certain oxidases such

as cyclooxygenases, lipoxygenases, dehydrogenases and peroxidases.

Free radical Reactions.

The free radicals damage the membranes and thereby change the receptor's alignments and ion channels. These changes finally affect the normal functioning of a cell by affecting the process of signal transduction.

Primary target of free radicals are unsaturated bonds in the lipids of membranes. This leads to the loss of membranes fluidity, receptor alignment and cellular lysis. It also attacks the sulphur containing enzymes resulting in the inactivation, cross linking and denaturation. Nucleic acids are also affected by the free radicals, Ultimate result of these actions are destruction of endothelial cells of blood vessels, macrophage invasion, suppressed immune response, inflammatory response, destruction of lung tissue, hastening of aging process and also impotency and infertility.

In a living system, lipid peroxidation is induced by free radicals and reactive oxygen species, which ultimately damage the cells, metals also induce LPO which may be of two types enzymatic and non-enzymatic. In the later case reducing agents, responsible for converting Fe^{+++} to Fe^{++} for further reaction are of chemical nature such as ascorbate, cysteine etc However, in the enzymatic mechanism, the reducing agent is an enzyme catalyzed reaction such as NADPH – cytochrome P-450 reductase. CCl_4 induced lipid peroxidation is also an example of enzymatic mechanism because here CCl_4 is metabolically activate to CCl_3^0 radical.

Free Radicals and Diseases:

Free radicals are involved in more than 50 diseases. Day to day researchers are adding more number of diseases to this list, In some cases it interacts with the progress of a disease along with other theological factors and in other cases the diseases are primarily due to free radicals.

Rheumatoid arthritis ischaemic and post ischaemic conditions, malignancy, mucocutaneous syndrome radiation effects, immune deficiency avitaminosis, aging and hypoxia are examples of such diseases where free radicals are involved in the pathology of several organs besides these disease, Alzheimer's disease, parkinsonism cerebral ischaemia are conditions where central nervous system is involved. Nephrotic syndrome, toxicity due to nonsteroidal anti-inflammatory drugs and metals, are diseases where free radicals attack the renal system. Other examples are atherosclerosis alcohol cardiomegaly, drug hepatitis, pollutant toxicity, tobacco toxicity cataract, retinopathy dermatitis, diabetes mellitus, ulcerative colitis etc.

I describes below briefly; some diseases which are being investigated in my laboratory for the past six years.

Free Radicals in Nervous diseases

Reactive Oxygen Intermediates, produced as a consequence of a physiological metabolic reactions, promote tissue injury which leads to brain trauma, ischemia, toxicity & neurodegenerative diseases.

Free Radicals and infertility

Reactive oxygen species are produced by living spermatozoa, its excess production on long persistence could be a significant cause of male infertility. They produce less motile sperms which are ineffective for

fertilization. The abnormal spermatozoa is a primary source of free radicals.

Free radicals in arthritis

Rheumatoid arthritis is recognized as a disease of oxidant stress. Infiltration of synovium by inflammatory cells lead to the formation of free radicals which causes extensive disruption of synovial membrane and promotes inflammatory tissue damage with cartilage and bone destruction.

Free radicals in Diabetes

free radical and oxidative stress are implicated in the pathogenesis of diabetes mellitus and its long term complications, In insulin dependent diabetes mellitus (IDDM), some environmental factors may play the role of initiator, in addition to genetic susceptibility.

Another etiology is immunological and toxic agents induce autoimmunity by causing antigen perturbation, As a result, cytotoxic antibodies are formed which cover the B-cells. This complex releases the chemotactic factors, which attract small lymphocytes, neutrophils and some granulocytes, these cells undergo respiratory burst and produce reactive oxygen intermediates (ROIs) such as O₂ superoxide radical anion, hydrogen peroxides, hydroxyl radical and singlet oxygen etc. These free radicals finally damage the B-cell and hamper the secretion of insulin secretion which leads to the onset of symptoms of Type I diabetes mellitus (IDDM).

Management of Diseases and Free Radical concept

Concept of Health in Ayurveda:

A living organism is the union of four components i.e Sense, Organ, Psyche and soul. It is the combination of three body humours, (Tridosh), seven basic tissues (Sapta dhatus) and three excretions (Trimalas). The five major elements of the body are panchmahaboota which are based on the existence in relation to nature and environment.

The union of four components, described above, are regulated by another three factors named as *Pran, Agni & Ojha*, *agni* is of 13 types. The one which is responsible for digestive power (*Jathragani*) is considered to be the most important

When *Pran, Agni & Ojha* are weakened, the interaction of three doshas is disturbed and it leads to the destabilization of the equilibrium of the body humours or disturbed normal physiology. These changes are manifested in the form of symptoms of a disease.

Disease process is the resultant of all the three factors i.e physical, psychic and environmental which operate simultaneously. There is a general concept of *Atiyoga* (excess), *Heeneyoga* (Less) or *Mithyayoga* (unwholesome use) of normal requirement as the root cause of all the diseases. The health practices can be visualized as nutrition, exercise, massage, rest, sleep, hygiene and follow up of daily routine to maintain the body humours. Nutrition is the critical determinant of immunocompetence and risk of illness, Undernourished individuals have impaired immune response which leads to more infections and immune related disorders. This nutritional deficiency includes protein-energy malnutrition, vitamin deficiency and shortage of minerals and coenzymes. All these factors have different mechanisms for affecting an individual and could be

counteracted by specific supplementation, some of these components directly affect the system and some have indirect effect by regulating the synthesis itself is reduced, therefore at this stage the diet having direct action becomes more important (direct effect).

Regarding the management of a disease, Ayurveda believes in the concept of natural healing of a disease by the regression of the causative factor (*Swabhavoparamvada*). The treatment part is bersons and the other which cures the diseases.

First group of medicines enhances the energy of an individual, delays ageing improves metabolism specially anabolic path ways, prevents from diseases, improves fertility ad sexuality upto old age.

In the modern concept, level of hormones and enzymes go down. Signaling mechanism is retarded and post translational changes in protein occur. These processes lead to the abnormal physiology resulting to the accumulation of toxic materials, unwanted body components, free radicals, reduced immunity, low vitality etc. Ubiquitous presence of free radicals have prompted the scientists to investigate their role in different biochemical pathways of a living system. Now it is well establishing that free radicals play an important role in regulating ageing process, memory, immune system, inflammatory disorders, diabetes, atherosclerosis, would healing, phagocytosis etc.

We all know Ayurveda is based on the triboshas concept of *Vata*, *Pitta* and *Kapha*. Although many vaidyas and modern scientists have tried to explain these terms in the scientific language but still it is not up to the perfection.

If we see out past normal ethos, habits, rules and regulations of different dharmas have always given preaching to keep the life health. Ayurveda has the holistic concept of treatment i.e. to concept of treatment i.e to consider t patient and its environment together while diagnosis & treating a patients.

But what is happening now. This modern civilization has led us to no where. This hurry, worry & curry have created several diseases, related to the metabolism such as diabetes, blood pressure, arthritis, Anxiety, depression etc. If you see, in young age or when the *Oja* is strong enough one can fight with the metabolic imbalances, created by the activities of this so called modern civilization but when oja goes down a new situation is created.

In Ayurveda this new situation is coined as the accumulation of doshas and in modern science one of the symptoms of this unwholesome habit is the excess generation of free radicals and the accumulation of the lipid peroxides such as lipofusins in the nerve cells.

Rasayana drugs and Antioxidants

Before describing the term “Antioxidants” it seems logical to discuss the term “Rasayana drug” of Ayurveda.

As per ‘Maharshi Charaka’, Rasayana is a group of medicines which basically vitalize a cell b opening all the srotas (systems), which are either blocked or partially open. Because of this basic property, these medicines are being used not only as a curative medicine for disease but also as the supportive medicine to almost every disease.

In other words, it can be safely said that medicines of Rasayana group can be given

in every disease wither alone or along with the main drug of that disease. At one hand, it cures a disease by itself and on the other hand it improves the efficacy of the other main drugs which are being given along with Rasayana. Its use is recommended from the adulthood with the objective to delay the aging process and to prevent the onset of other old age metabolic disorders.

Similar is the case with the use of antioxidants. They are being recommended in all types of diseases and even for general health. Nowadays people are taking antioxidants as the diet supplement to prevent the process of aging and old age diseases.

What are Antioxidants?

Those agents which lower the burden of free radicals are known as antioxidants. They have different classifications based on their site of action in the free radical chain reaction. They could be divided into three groups.

- (a) Inhibitors,
- (b) Chain breakers,
- (c) Chain terminators.

Based on the structural specifications, they are divided into

- (a) Enzymatic
- (b) Non-Enzymatic (Natural or Synthetic).

One functional basis antioxidants could be further grouped under two main categories.

- (i) The one which prevents the generation of free radicals and
- (ii) The other, which intercepts the free radicals, already generated.

The preventive defences include efficiency of electron transfer and sequestration of transition metal ions. Other form of prevention is the removal of superoxides and peroxides (H_2O_2 and lipid hydroperoxides) that react with transition metal ions to produce reactive free radicals, superoxides dismutase, catalase and glutathione peroxidase are examples of this group. Antioxidants exist in membrane as well as in aqueous compartments.

Pharmacological antioxidants have their specific action. For example allopurinol and folic acid inhibit xanthine oxidase. Soybean trypsin inhibitors phenyl methylsulfonyl fluoride (PMSF) are protease inhibitors, adenosine, local anaesthetics, calcium channel blockers etc inhibit superoxide generation by inhibiting NADPH oxidase.

Some non- enzymatic free radical scavengers are mannitol, DMSO, Dimethyl thiourea (DMTU) which scavenge OH radical. Other examples are spin traps, bilirubin, urate, glutathione, 17-aminosteroids and albumin. Ceruloplasmin, transferrin and desferrioxamine are other antioxidant which inhibit the iron redox cycling.

Some antioxidants act through augmenting the endogenous antioxidant activity such as selen and acetylcysteine which act by enhancing glutathione peroxidase activity. Third category of natural antioxidant defence is the repair process, which removes damaged biomolecules before they can accumulate and alter cell metabolism.

A great deal of efforts are being made on finding out the effective antioxidant drugs for the management of free radical diseases, such as 'Probuco' Phytochemicals have a significant part to play in the management of such diseases.

Mechanism of action: Various classes of phytochemicals have been shown to have antioxidant property. They have different mechanisms of action and act at different sites in the chain reaction. The activity of natural products- antioxidants is due to the presence of substituted groups such as carbonyl, phenolic, phytol side chain, electron withdrawing group electron donating group etc. They may be phenolic or on phenolic.

In phenolics, the number and position of phenolic groups decide the antioxidant potential of a compound. Here hydroxyl group donates hydrogen to radicals which are converted to a stable non-radical product and the chain propagation is terminated. Phenolic group at para position enhances the antioxidant property.

Non – phenolic compounds participate in the antioxidant mechanism through electron transfer & resonance stabilization process. The quinone acts as electron acceptor and prevents free radical chain reaction. Alkaloids, such as strychnine, Engenol, Withaperuvin E, Nonberginine, Benzylisoquinoline; Flavonoids as sylibin, Anthraquinones as Rubiadin, Emodin. Etc. are a few examples. Synthetic antioxidants include parabenzoquinone, probucol etc. Physiological enzyme based reduction of

these quinones are caused by xanthine oxidase, cytochrome p-450 reductase, resulting to the formation of semiquinone and hydroquinone.

Natural products, which possess a factor favouring quinone formation, or the presence of an electron withdrawing group at para position are reported to be good antioxidants. Vitamin E, Vitamin A (Beta carotene) and Vitamin C are important components of this family which interfere at the level of propagation. Glutathione, is another important antioxidant in the system. It undergoes oxidation and reduction under enzymatic control and inactivates free radicals.

With this basic similarity in Rasayana drugs of Ayurveda and antioxidants of modern medicine, we have investigated the effect of several Ayurvedic medicines. Some of them are *Rubia cordifolia*, *strychnos nuxvomica*, *Moringa oleifera*, *semecarpus anacardium*, *Mucuna pruriens*, *Bacopa monnieri*, *Nardostachys jatamansi* etc. We have observed that these medicinal plants have anti-oxidant property and can be used as a medicine to manage the free radical mediated diseases. Of course, their active fraction could be more effective and with lesser side effects. These observations support the basic theory of Ayurveda.

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