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AN OVERVIEW FREE RADICALS AND ANTIOXIDANTS IN THE CONTEXT OF AYURVEDA & CONTEMPORARY SCIENCE

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ABSTRACT
In India the Ayurvedicschool of medicine is possibly the first to designate the science of life, its central theme being efforts to shield life from disease and aging. Accordingly Ayurveda situates a considerable thought on the science of Gerontology containing Rasayana Therapy. The prolongation of life is closely related with profound economical vagaries as well as with the changes of social structure. Each of these variations is studies under the special disciplines of Geriatrics and Gerontology. Ayurveda is known to deliberate the phenomenon of aging from entirely a new angle.Free radicals are atoms, ions or molecules that contain an unpaired electron. Thus, they become electrically charged because number of negatively charged electron does not match with positively charged protons. When a molecule loses or gains a single electron in its outer orbit, it becomes free radical. In fact, a free radical is defined as 'a molecule that can exist independently for a period of time with one or more unpaired electrons'. There are mainly two sources of free radicals that is Exogenous & Endogenous. Many types of free radical found in our body that is Superoxide radical, Hydrogen peroxide, Hydroxyl radical, Nitric Oxide (NO) radical, Carbon tetrachloride (CCl4) and Singlet oxygen. Free radicles can attack vital cell components like polyunsaturated fatty acids (PUFA), proteins, and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross linking, inhibition of protein synthesis, DNA damage; ultimately resulting in cell death. Some of the wellknown consequences of generation of the free radicals in vivo are. DNA strand scission, nucleic acid base modification, protein oxidation and lipid peroxidation. In Ayurveda, Rasayana are acting like Antioxidant.

KEYWORDS: Free radicals, Antioxidants, Ayurveda, contemporary.

1. INTRODUCTION
In India the Ayurvedicschool of medicine is possibly the first to designate the science of life, its central theme being efforts to shield life from disease and aging. Accordingly Ayurveda situates a considerable thought on the science of Gerontology containing Rasayana Therapy. The prolongation of life is closely related with profound economical vagaries as well as with the changes of social structure. Each of these variations is studies under the special disciplines of Geriatrics and Gerontology. Ayurveda is known to deliberate the phenomenon of aging from entirely a new angle. In Ayurveda, Rasayan can compare with Antioxidant. When all the medical researchers were scratching their heads for a plausible explanation regarding ageing process, a scientist hit the headlines with his new concept in mid 50s of the twentieth century which got worldwide attraction. It has been considered as the biggest advancement in the field of medical science next to Louis Pasteur's discovery of 'germs' as the cause of human diseases. This is nothing but *'free radical concept'* which, today, known to be involved in the pathophysiology of as many as eighty diseases such as arthritis, atherosclerosis, cancer etc. Free radicals are atoms, ions or molecules that contain an unpaired electron. Thus, they become electrically charged because number of negatively charged electron does not match with positively charged protons. RasayanaTantrais one of the eight major clinical discipline of AstangaAyurveda. The term does not only denote to a drug or a therapy but to ainclusive discipline which may of course embrace a therapy. It is a multiangledmethodology taking care of the body, the mind and the spirit, thus affording a total well being of distinct. Rasayanatherapy i.e the rejuvenation therapy affords a comprehensive physiologic and metabolic restoration as is evident from

the fundamental statement of Carakai.e. **"Labhopayo hi sastanamrasadinamrasaynam".** Ca, Ci:1.Rasayan is having antioxidant property which removes free radicals from body.

2. AIMS & OBJECTIVES

- ✓ To know free radicles in the context of Ayurveda & contemporary Science
- ✓ To Know Antioxidants in the context of Ayurveda & contemporary Science

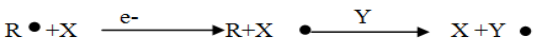
3. FREE RADICALS

It all began way back in 1954 at the Donner Laboratory of Medical Physics of the University of California, Berkeley by an eminent researcher *Dr. Denham Harman.* The, working on the subject of ageing proposed *'Free radical theory of ageing'*, which first stated free radicals as the cause of degenerative changes in the body leading to ageing. This was first published in July 14, 1955 by the University of California Radiation Laboratory Report titled *"Ageing: a theory based on free radical and radiation chemistry"* and as a article a year later in the journal of Gerontology. His first talk *'Ageing: the theory based on free radical and radiation chemistry with application to cancer and atherosclerosis'* was presented on February 6, 1956 as a Donner LaboratorySeminar.

3.1 General description of free radicles

Free radicals are atoms, ions or molecules that contain an unpaired electron. Thus, they become electrically charged because number of negatively charged electron does not match with positively charged protons.

When a molecule loses or gains a single electron in its outer orbit, it becomes free radical. In fact, a free radical is defined as *'a molecule that can exist independently for a period of time with one or more unpaired electrons'*. Electron imbalance causes a free radical to seize an electron from surrounding molecules to form a complete pair. The donor molecule, by giving up an electron, may itself become a radical. The chain reaction thus triggered can damage cell membranes and lead to disease.



The reaction of one free radical with another free radical will, in general, terminate the chain of free radical reactions.

Conventionally, the radicals are denoted by dot (●).

Oxygen is vital for aerobic life processes. In normal health, almost 98% of oxygen used by cells to burn food are converted into water by the mitochondrial cytochrome oxidase system. Rest of the oxygen that escapes from the cells energy engines is converted into 'reactive oxygen species' (ROS) by univalent reduction of oxygen. Oxygen is frequently referred to as a *'reactive oxygen species'* based on its ability to take on electrons (reduction) or to give up electrons (oxidation). ROS include not only O_2 centered radicals such as superoxide ($O^{\bullet-}_2$), hydroxyl ($OH^{\bullet-}$), nitric oxide radical ($NO^{\bullet}$) and peroxyl radical ($RO^{\bullet}_2$) but also some reactive non radicals derived from O_2 such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen (O_2) and ozone (O_3).

Thus, the cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. When the balance between ROS production and antioxidant defence is lost, i.e., excessive production of ROS beyond the antioxidant defence capacity of the cell occurs, *'oxidative stress'* sets in which through a series of events deregulates the cell physiology leading to various pathological conditions including cardiovascular dysfunction, neurodegenerative disorders, gastroduodenal pathogenesis, metabolic dysfunction of almost all vital organs cancer and *prematureageing*.

The free radical mediated oxidative stress results in oxidation of membrane lipoproteins, glycoxidation and oxidation of DNA, subsequently cell death ensues. ROS from damaged cells also attack the adjacent cells, resulting ultimately in cell injury. Furthermore, cell injury itself has been reported to cause severe oxidative stresses leading to disorganization of cell structure and function.

3.2 Sources of free radicals (oxidants)

There are mainly two sources viz.

(1) Exogenous
(2) Endogenous

Exogenous sources include air pollution (of which industrial waste and cigarette smoke are major contributors) radiation, drugs and pathogens. Trace metals, notably lead, mercury, iron and copper are also major sources of free radical generation. Normal diets containing plant foods with large quantities of certain compounds such as phenols and even caffeine may contribute to the exogenous supply of oxidants to the body.

Endogenous free radicals are produced in the body by different mechanisms. First, from the normal metabolism of oxygen requiring nutrients.

Mitochondria - the intracellular powerhouses which produce the universal energy molecule, adenosine triphosphate (ATP) normally consume oxygen in this process and convert it to water. However, unwanted byproducts such as the superoxide anion, hydrogen peroxide and the hydroxyl radical are inevitably produced, due to incomplete reduction of the oxygen molecule. It has been estimated that more than 20 billion molecules of oxidants per day are produced by each cell during normal metabolism.

Second, white blood cells destroy parasites, bacteria and viruses by using oxidants such as nitric oxide, superoxide and hydrogen peroxide. Consequently, chronic infections result in prolonged phagocytic activity and increased exposure of body tissues to the oxidants.

Third, other cellular components called peroxisomes produce hydrogen peroxide as a byproduct of the degradation of fatty acids and other molecules. In contrast to the mitochondria which oxidise fatty acids to produce ATP and water, peroxisomes oxidise fatty acids to produce heat and hydrogen peroxide. The peroxide is then degraded by an enzymatic antioxidant calledcatalase. Finally, an enzyme in the cells called cytochrome P450 is one of the body's primary defences against toxic chemical ingested with food. However, the induction of these enzymes to prevent damage by toxic foreign chemicals like drugs andpesticides also results in the production of oxidant byproducts.

3.3 Types of free radicals:

Though a variety of free radicals are produced (approximately 20 billion molecules) every day, total number of them is not yet known. Some of them are bit elaborated here.

Although, O_2 can behave like a radical owing to the presence of two unpaired electrons of parallel spin, it does not exhibit extreme reactivity due to quantum mechanical restrictions. Its electronic structure results in the formation of water by reduction with four electrons i.e.,

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O.$$

In the sequential univalent process by which O_2 undergoes reduction, several reactive intermediates are formed, such as superoxide (O_2), hydrogen peroxide (H_2O_2) and the eremely reactive hydroxyl radical (OH); collectively termed as the
reactive oxygen species (ROS). The process can be represented as:

$$O_2 \xrightarrow{e^-} O_2^{\cdot-} \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} OH^{\cdot} \xrightarrow{e^-} H_2O$$

3.3.1 Superoxideradical

For the production of $O^{\cdot-}$, normally the tendency of univalent reduction of O_2 in respiring cells is restricted by cytochrome oxidase of the mitochondrial electron transport chain, which reduces O_2 by four electrons to H_2O without releasing either O^- or H O . However, O^- is invariably produced in respiring cells. This is due to probable 'leak' of single electron at the specific site of the mitochondrial electron transport chain, resulting in inappropriate single electron reduction of oxygen to O^- . When the electron transport chain is highly reduced, and the respiratory rate is dependent on ADP availability 'leakage' of electrons, at the ubisemiquinone and ubiquinone sites increase ssoasto result in production of O^- and H_2O_2.

$$O_2 \xrightarrow{e^-} O_2$$

O_2^- is toxic to a cell growing under aerobic conditions. O_2^- shows high reactivity in hydrophobic environments, but poor reactivity in aqueous solutions. Due to its charged state, it can not cross a biological membrane with the exception of erythrocyte membrane which has an 'anion channel' that helps in the crossing of O_2^-. An indirect deleterious action of O_2^- is mediated by its dismutation to H_2O_2, which is sensitive tocatalase.

3.3.2 Hydrogen peroxide

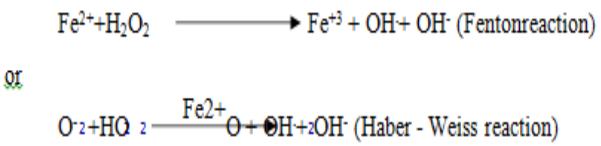
For the production of H_2O_2, peroxisomal oxidases and flavoproteins, as well as D-amino acid oxidase, L-hydroxy acid oxidase and fatty acyl oxidase participate. Cytochrome P450, P450 reductase and cytochrome b5 reductase in the endoplasmic reticulum under certain conditions generate O_2^- and H_2O_2 during their catalytic cycles. Likewise, the catalytic cycle of xanthine oxidase i.e., xanthine oxidase - produced by proteolytic cleavage of xanthine dehydrogenase (during ischaemia - upon reperfusion in presence of O_2) acts on xanthine or hypoxanthine to generate O_2^- and H_2O_2. The phagocytic cells, such as neutrophils, when activated during phagocytosis, generate O_2^- and H_2O_2 through activation of NADPH oxidase. In addition, spontaneous dismutation of O_2^- at neutral pH or dismutation by superoxide dismutase results in H_2O_2 production.

$$O_2^{\cdot-} + 2H \xrightarrow{e^-} H_2O_2.$$

In a transition metal free system, H_2O_2 shows limited toxicity. However, since it is long lived and membrane permeable, it may diffuse considerable distance away from its site of generation.

3.3.3 Hydroxyl radical

The hydroxyl radical is the most reactive oxygen radical known to chemistry till date. It has tremendous potential for causing biological damage, since it attacks all biological molecules as soon as it comes in contact with them, setting off free radical chain reactions. They can attack all constituents of DNA. Since OH reacts very quickly with everything, it does not last long enough in the body to migrate elsewhere i.e. it has very limited diffusion capacity. Thus, the extent of damage to the cells by O_2^- and H_2O_2 increases in presence of the transition metal ions due to the generation of more powerful $OH^{\cdot}$.For the production of OH, except during abnormal exposure to ionising radiation, generation of $OH^{\cdot}$*in vivo* requires the presence of trace amount of transition metals like iron or copper. A simple mixture of H_2O_2 and Fe^{2+} salt forms $OH^{\cdot}$.



However,redoxactivefreeironorcopperdonotexistinbiological systems.

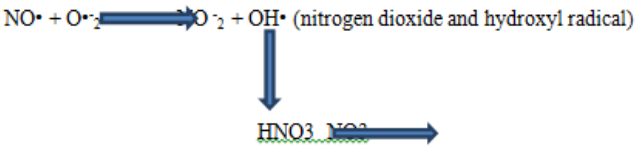
As these transition metal ions remain bound to proteins, membranes, nucleic acid or low molecular weight chelating agents like citrate, histadine or ATP. During ischaemic condition and cellular acidosis transition metal ions may be released from some metalloproteins, resulting in the generation of OH.

3.3.4 Nitric Oxide (NO) radical

It is a soluble free radical gas that is produced not only by endothelial cells but also by macrophages and specific neurons in the brain. It is synthesized from L- arginine, molecular oxygen and NADPH by the enzyme nitric oxide synthase (NOS).

Since the *in vivo* half life of NO is only a matter of seconds, the gas acts only on cells in close proximity to where it is produced. The NO produced by macrophages, acting as a free radical, is cytotoxic to certain microbes and tumours cells. It can oxidise sulfhydryl groups on proteins and cause a depletion of cytosolic glutathione, and it can react with superoxide anion to form the strong oxidant nitrogen dioxide ($NO^{\square -}$) and highly reactive hydroxyl radical as well as peroxynitrite anion (NO^-).

(NO•-2) and highly reactive hydroxyl radical as well as peroxynitrite anion (NO-3).



3.3.5 Carbon tetrachloride (CCl_4) is used widely in dry cleaning industry. The toxic effect of CCl_4 is due to its conversion by P450 to the highly reactive toxic free radical $CCl^{\cdot}$ which initiates lipidperoxidation.

3.3.6 Singlet oxygen

It is possible to convert the slow reacting oxygen to fast reacting, powerful oxidising agent by the simple rearrangement of its electrons called singlet oxygen. This type of conversion can be seen in photosensitization reaction.

4. FREE RADICALS STIMULATE CELLULAR DAMAGE

Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids (PUFA), proteins, and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross linking, inhibition of protein synthesis, DNA damage; ultimately resulting in cell death. Some of the wellknown consequences of generation of the free radicals *in vivo* are. DNA strand scission, nucleic acid base modification, protein oxidation and lipid peroxidation.

Lipid peroxidation

Free radicals in the presence of oxygen may cause peroxidation of lipids within plasma and organellar membranes. The presence of double bond adjacent to a methylene group makes the methylene C-H bonds of polyunsaturated fatty acid (PUFA) weaker and therefore the hydrogen becomes more prone to abstraction. While lipid peroxidation is not initiated by O_2 and H_2O_2, hydroxyl ($OH^{\square}$), alkoxy radicals ($RO^{\square}$) and peroxy radicals (ROO) result in initiating the lipid peroxidation. This can lead to a self perpetuating process since peroxy radicals are both reaction initiators as well as the products of lipid peroxidation. Lipid peroxy radicals react with other lipids, proteins and nucleic acids, propagating thereby the transfer of electrons and bringing about the oxidation of substrates. Cell membranes, which arestructurally made up of large amounts of PUFA, are highly susceptible to oxidative attack and consequently, changes in membrane fluidity, permeability and cellular metabolic functions result.

DNA damages

ROS can cause oxidative damages to DNA, both nuclear and mitochondrial. The nature of damage include mainly base modification, deoxyribose oxidation, strand breakage and DNA protien cross links. Among the various ROS, $OH^{\square}$ generates various products from the DNA bases which mainly include C-8 hydroxylation of guanine to form 8-oxo-7, 8-dehydro-2'-droxyguanosine, a ring opened product; 2,6-diamino-4-hydroxy-5-formamino dipyrimidine, 8-OH-adenine, 2-OH-adenine, thymine glycol, cytosine glycol, etc. Mutation arising from selective modification of G:C sites specially indicates oxidative attack on DNA by ROS. ROS may interfere with normal cell signalling, resulting thereby in alteration of the gene expression, and development of cancer by redox regulation of transcriptional factors/activator and/or by oxidatively modulating the protein kinase cascades.

The oxidative damage of mitochondrial DNA also involves base modifcations and strand breaks, which leads to formation of abnormal components of the electron transport chain. This results in the generation of more ROS through increased leakage of electrons and therefore further damage. Oxidative damage to

mitochondrial DNA may promote cancer and ageingeventually.

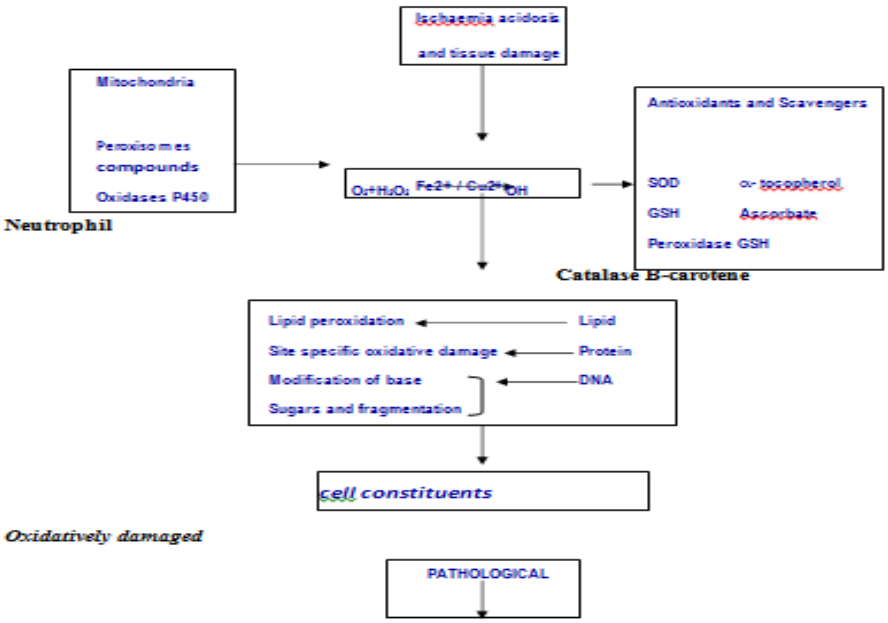
Oxidative damage of proteins

During mitochondrial electron transport chain, free radicals are produced which can stimulate protein degradation. Oxidative protein damage may be brought by metabolic processes which degrade a damaged protein to promote synthesis of a new protein. Lipofuscin, an aggregate of peroxidised lipid and proteins, accumulates in lysosomes of aged cells. On the basis of extensive studies on ageing processes, it has been established that catalytically inactive or less active, more thermolabile forms of enzyme accumulate in cells during ageing and show a dramatic incrase in the level of protein carbonyl content; an index of metal catalysed oxidation of proteins. (In human, erythrocytes, levels of glyceraldehyde 3-P-dehydrogenase, aspartate aminotransferase and phosphoglycerate kinase decline with age together with an increase in protein carbonyl content. The carbonyl content of protein in rat hepatocytes also increases with age along with decrease in the activities of glutamine synthetase and glucose 6-P-dehydrogenase, without any loss in the total enzyme protein. An age related oxidative modification of human ceruloplasmin, a copper containing protein in human plasma has also beenreported.

The mechanism of oxidative damage of proteins by ROS has been studied *in vitro* by generating these reactive, species either in solution or 'site specifically' within the protein. While the former damage is termed as nonspecific (global), the latter damage is termed as site specific (localized) damage. Nonspecific damage can be stimulated by generating activated oxygen species *(in situ)*, which lead to aggregation and fragmentation of the protein and modification of almost all the amino acid residues. In contrast, localised damage can occur when the ROS, such as $OH^{\bullet}$ are formed at putative metal binding sites in proteins. When these sites are occupied by iron or copper, they can in the presence of suitable reductants, O^{-}_{2} or ascorbate, react with H_2O_2 to generate highly reactive $OH^{\bullet}$ which reacts preferably with specific amino acids present in the vicinity of the metal binding site, inducing thereby specific damage that shows no gross structural modification. The concept of 'site specificity' is as follows: (i) catalytic metal ions, such as iron or copper would be bound to the target molecule (protein, DNA or cell membrane) and the $OH^{\bullet}$ produced by O^{-}_{2} (or ascorbate)_ and H_2O_2 produced at the iron or copper binding site would then react preferentially with the target molecule. (ii) the damaging effect of $OH^{\bullet}$ is observed at a specific site where catalytic ions are bound and (iii) the defensive action of the free radical scavengers to remove $OH^{\bullet}$ from the specific site decreases dramatically, since they are unable to access the microenvironment. Ceruloplasmin, albumin and angiotensin are copper containing proteins which undergo site specific oxidative damage on being exposed to ascrobate. Recently, gastric peroxidase has also been shown to undergo site specific oxidatived damage with the loss of two lysineresidues, upon the exposure of the enzyme toascorbate.

Cu^{2+}- H_2O_2 system, leading to generation of $OH^{\bullet}$ at the putative Cu^{2+} binding site of the enzyme.

Overall picture of the metabolism of ROS and the mechanism of oxidative tissue damage.



5. REACTIVE OXYGEN SPECIES (ROS) AND AGEING

It is now generally agreed that ageing and age related diseases result from ROS mediated oxidative damage of lipid, protein and nuclear and mitochondrial DNA molecule. The concentration of oxidatively damaged proteins, lipids and DNA has been reported to increase with age. The hydroxyl and peroxy radicals cause extensive damage of proteins resulting in ageing and age related degenerative diseases. Other than O^- and H O mediated oxidative damage, mutation in mitochondrial DNA also leads to the formation of defective respiratory enzymes which not only result in decreased ATP synthesis but also generate more ROS to cause further oxidative damage. This vicious cycle is mainly responsible for ageing and age related disorders. Melatonin, a pineal hormone, having antioxidant property, declines significantly with the increase in age. This decline in melatonin coincides with the increased oxidative damage andpathogenesis.

6. ANTIOXIDANT DEFENSEMECHANISM

Everyday approximately 20 million molecules of free radicals are produced. If these free radicals attack altogether, then living organism fails to exist. Each cell protects itself from damage by producing free radical scavengers such as enzymes that neutralise free radical. Thus, balance is maintained between free radical production and scavenging activity of enzymes. This is known as Antioxidant defence system.

Antioxidants are defined as *'substances whose presence in relatively low concentrations significantly inhibits the rate of oxidation of targets'*.

Being present in serum, these antioxidants circumvent the damage caused by oxygen free radical. They consist of substances that provide the much needed stability to the free radical by allowing the pairing of electrons. Thus, they counteract the free radicalattack.

7. TYPES OF ANTIOXIDANT ACTIVITY

It may be accomplished by three different mechanisms:

- ✓ By inhibiting the generation of ROS.
- ✓ By directly scavenging the free radicals by means of anti radical scavenging enzymes such as SOD, catalase and glutathioneperoxidase.
- ✓ By raising endogenous antioxidant defences i.e. unregulated expressions of the genes encode the enzymes SOD, catalase or GSH -Px

This antioxidant defence system is basically of two types.

(i) *Primary defence*
(ii) Secondary defence

1. Primarydefence

This is again subdivided into two types -

(a) Antioxidant nutrients
(b) Antioxidant scavengingenzymes

Antioxidant nutrients: Antioxidant defences rely heavily on vitamins and minerals from the diet. These include beta carotene (precursor of Vit. A), Vitamin E (□-tocopherol), vitamin C (ascorbic acid), selenium, zinc, manganese and copper.

Antioxidant scavenging enzymes: Superoxide dismutase (SOD), catalase and peroxidases form defence system against ROS. While SOD lowers the steady state level of O^- , catalase and peroxidases do the same for H O.

(a) **Superoxide dismutase (SOD):** The first enzyme involved ion the antioxidant defence. It is a metalloprotein found in both prokaryotic and eukaryotic cells. The iron containing (Fe-SOD) and the manganese containing (Mn-SOD) enzymes are characteristic of prokaryotes. In eukaryotic cells, the predominant forms are the copper containing enzyme and the zinc containing enyzme, located in the cytosol. The manganese containing SOD is found in the mitochondrialmatrix.

The metals bound to SOD catalyse the reaction of the O^-_2 molecules with H^+ ions to form H_2O and O_2. SOD accelerates this reaction by ten thousand times. The Manganese containing (Mn-SOD) in mitochondria presumably removes O_2produced as a result of electron leakage on the O_2 from the mitochondrial electron transport chain and by mitochondrial oxidase enzymes. The copper and zinc containing SOD (Cu-SOD and Zn-SOD) deals with O_2 from the cytochrome P450 enzymes, which are located in the endoplasmic reticulum of the cell. Some Cu-SOD and Zn-SOD may be present in the nucleus and some are present in peroxisomes.

(b) ***Catalase:*** Catalase present in almost all the cells is localised in the peroxisomes or the microperoxisomes. It is a haemoprotein and catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and□.

(c) ***Heme peroxidase:*** Hemeperoxidases such as horseradish peroxidase, lactoperoxidasecatalyse the oxidation of a wide variety of electron donors with the help of H_2O_2 and thereby scavenges the endogenousH_2O_2.

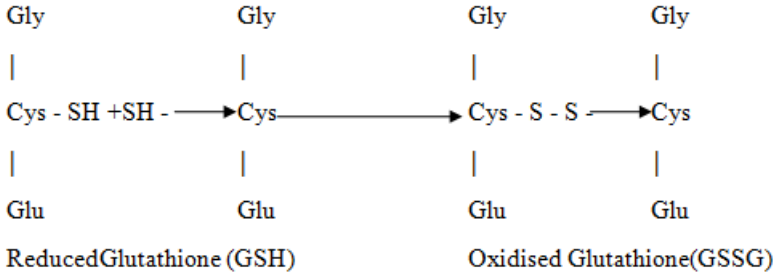
(d) ***Glutathione peroxidase:*** It catalyses the reaction of hydroperoxides with reduced glutathione (GSH) to form glutathione disulphide (GSSG) and the reduction produce of the hydroperoxide. This enzyme is specific for its hydrogen donor, GSH and nonspecific for the hydroperoxides ranging from H_2O_2 to organic hydroperoxides. It is a selenoenzyme; two third of which is present in cytosol and one third inmitochondria.

Glutathione peroxidases remove H_2O_2 at a high rate by using it to oxidise reduced glutathione (GSH) into oxidised glutathione (GSSG).,

$$2\,GSH + H_2O_2 \xrightarrow{GSSG} 2H_2O$$

GSH is made up of 3 amino acids (glutamic acid, cysteine and glyucine) joined together (a tripeptide).

GSSG is made by joining two reduced glutathione molecules by their -SH groups, losing the two hydrogens and forming a disulphide bridge.



The GSSG must then be converted back to GSH, which is the function of glutathione reductase, an enzyme containing FAD (a derivative of the water soluble vitamin riboflavin, vitamin B_2)

$$GSSG + NADPH + H^+ \longrightarrow NADP^+ + 2GSH$$

NADPH (a molecule resembling NADH) is used by glutathione reductase as a source of reducing power.

Reduced glutathione (GSH) is one of the most potent biological molecules that affect scavenging functions in the biological system. In addition to the role as an essential cofactor for certain enzymes, GSH performs the function of maintaining cellular integrity by virtue of its redox properties. The utilization of GSH for detoxification places a high demand on GSH in the liver. Hepatic GSH usually declines during metabolism of foreign compounds which require the thiol for bio-transformation. The three enzymes of GSH peroxidase system such as GSH peroxidase, GSH reductase and glucose - 6 - phosphate dehydrogenase (G6PD) supplying reducing equivalents needed for GSH reductase activity, which in turn maintains adequate concentration of GSH required for GSH peroxidase activity.Changes in tissue GSH and blood levels of GSH are possibly related to changes in either its synthesis or utilization (Cora et. al., 1987). The scavenging function of free radicals are efficiently carried out by antioxidant defence enzymes through glutathione redox cycle.

2. Secondary Defence

In addition to the primary defence against ROS by antioxidant enzymes, secondary defence against ROS is also offered by small molecules which react with radicals to produce another radical compound, the 'scavengers'. When these scavengers produce a lesser harmful radical species, they are called 'antioxidants' - tocopherol, ascorbate and reduced glutathione (GSH) may act in combination to act as cellular antioxidants. -tocopherol, present in the cell membrane and plasma lipoproteins functions as a chain breaking antioxidant. Once the tocopherol radical is formed, it can migrate to the membrane surface and is reconverted to -tocopherol by reaction with ascorbate or GSH. The resulting ascorbate radical can regenerate ascorbate by reduction with GSH, which can also directly scavenge ROS and the resulting GSSG can regenerate GSH through NADPH glutathione reductase system.

Since ROS mediated oxidative stress is now regarded as the major factor causing ageing and age related neuro-degenerative diseases, suitable antioxidant therapies to control these processes have already attracted world wide attention in recent years. The pineal hormone, melatonin, having potent antioxidant activity is a potentially promising candidate for the control of ageing and other ROS mediated pathogenesis.Restricting the caloric intake has also been shown to delay ageing through (i) decreased production of mitochondrial O^-_2 and H_2O_2 and (ii) increased production of antioxidant defences, leading thereby to decreased production of oxidatively damaged proteins, lipids and DNA. Caloric restriction may thus decrease the oxidative stress and damage and may prolong life inhumans.Isolation of an antioxidant factor which is specific in its action, is nontoxic, and shows antistress property, from the natural sources such as plants and the therapeutic application of such an antioxidant factor would perhaps be one of the better approaches to control the ROS mediatedpathogenesis.

8. RASAYAN

RasayanaTantrais one of the eight chief clinical disciplines of AstangaAyurveda. The term does not only represent to a drug or a therapy but to a inclusive discipline which may of course embrace a therapy. It is a multi angledmethodcaptivating care of the body, the mind and the spirit, thus affording a total well being of distinct. Rasayanatherapy i.e the rejuvenation therapy affords a comprehensive physiologic and metabolic restoration as is evident from the fundamental statement of Carakai. e. “Labhopayo hi sastanamrasadinamrasaynam”. Ca, Ci:1. Rasayan is having antioxidant property which removes free radicals from body. Ayurveda has two main objectives – a) To maintain the health of healthy individuals. b) To cure the diseases of diseased persons. Rasayanacikitsais mainly used for maintaining the health of healthy individuals although it can be used for diseased also. Ayurveda and other Indian traditions deliberated the total span of life

over one hundred years. The vedic hymns proclaim an active, healthy life of hundred years – "**JivemSaradahSatam, PasyemSaradahSatam" etc. Isavasyaupanised, Aitaraiya. Brahaman, Kathopanisad, vagbhatta** and carakadescribe an active life span of hundred years.

The word Rasayanacontains of two words viz,
(1) Rasa
(2) Ayana.
The word Rasa states to the Rasa Dhatusin the perspective of RasadiSaptaDhatusand to the pharmacodynamic properties of a drug in the context of Rasa Gunaetc. Ayanameans flow i.e. the measures by which one is adept of getting the nourishing Rasa. Thus Rasaynais that process by which all the body tissues are encouraged. Consequently Rasayanasupports in regeneration, revival and revitalization of Dhatus. Rasayanadrugs and measures deed at one or all of the three levels. As a result rich, good and healthy Dhatusare delivered in the body. This stretches to a person Longevity, Immunity, Vitality, Happiness, Improved intellect etc. 1) Acting at all levels of Rasa by enriching its nutritional value of the circulating, plasma, Examples – Draksha, milk,Satavari, Salparnietc. 2) Acting at the level of Agni i.e at the level of digestion and metabolism. Acting at the level of Srotamsii.e at the microcirculatory channels carrying nutrition to the tissues. These Rasayanaswipes and activate the micro – circulatory channels i.e. Srotosuddhiprincipal to improved tissue health and their quality. Example – Guggulu,Pippali, Rasonaetc.Classification of Rasayana A comprehensive classification of Rasayanais the contribution of Susruta. Further enhancement was done by Dalhana.

Following is the most rational classification of Rasayanaaccording to the textual descriptions and commentaries there upon.

A) According to mode of administration (Carak)
i. Vatatapika / SouryaMarutika(For purpose of outdoor)
ii. KutiPravesika(For purpose of indoor)

B) According to object (Susruta)
i. KamyaRasayana
a. PranaKamya(Promotes longevity and life span)
b. MedhaKamya(Improves mental faculties)
c. SreeKamya(Improves luster of the body)
ii. NaimittikaRasayan
iii. AjasrikaRasayana

C) Specific RasayanaDrugs and Specific Activities
i. MedhyaRasayana
ii. AcaraRasayanaBuddhiMedhaVardhaka
b. AyuVardhak

D) According to Prabhava(Effect)
i. SamsodhanaRasayanaii. SamsamanaRasayana.

9. CONCLUSION

Free radicals are atoms, ions or molecules that contain an unpaired electron. Thus, they become electrically charged because number of negatively charged electron does not match with positively charged protons. When a molecule loses or gains a single electron in its outer orbit, it becomes free radical. In fact, a free radical is defined as *'a molecule that can exist independently for a period of time with one or more unpaired electrons'.* There are mainly two sources of free radicals that is Exogenous & Endogenous. Many types of free radical found in our body that is *Superoxide radical, Hydrogen peroxide, Hydroxyl radical, Nitric Oxide (NO) radical, Carbon tetrachloride (CCl4) and Singlet oxygen.* Free radicles can attack vital cell components like polyunsaturated fatty acids (PUFA), proteins, and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross linking, inhibition of protein synthesis, DNA damage; ultimately resulting in cell death. Some of the wellknown consequences of generation of the free radicals *in vivo* are. DNA strand scission, nucleic acid base modification, protein oxidation and lipid peroxidation. In Ayurveda, We are using Rasayan as a Antioxidant.