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Oxidative Stress: A Review

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Abstract:
Oxidative stress is imbalance between the pro oxidant and antioxidant status in the living organisms. In human, reactive oxygen species (ROS) and free radicals are produced in various metabolic activity and immune response under normal physiological condition. Alteration or increase in ROS and free radicals generation leads to oxidative stress, which is responsible for disease condition such as atherosclerosis, cancer, Parkinson's disease, Alzheimer's disease; alcohol induced liver disease, cardiovascular disease and so forth. Thus, ROS and free radicals generation have both protective as well as harmful effect. Antioxidants are those substances which scavenge the ROS and free radicals and protect the different biomolecules such as DNA, protein, carbohydrates, lipid and so forth. In this review, we focus on the production of ROS, oxidative stress and scavenging properties of antioxidants.

Keywords: *Reactive oxygen species, antioxidants, oxidative stress*

1. Introduction

Oxidative stress refers to the imbalance between the production of reactive species and antioxidant defence. According to Sies oxidative stress is "a disturbance in the pro oxidant-antioxidant balances in favour of the former, leading to serious damage" [1]. Oxidative stress focuses the attention of worldwide researchers for its damaging effects on the human body [2]. and essential for life and also responsible for the death of a cell. In organisms including human's reactive oxygen species (ROS) and free radicals are produced during metabolic and immune system function. Molecular oxygen (O_2) has ability to un-pair and leave free radicals which are unstable and highly reactive leads to formation of ROS [1, 3]. When the concentration of ROS beyond a certain limit it is beneficial as biological functions such as phagocytosis, apoptosis, necrosis, and provide protection against pathogens. In oxidation reaction several enzymes (peroxidase) uses H_2O_2 as a substrate which involving the synthesis of complex organic molecules in the organisms [2,3]. Body have defensive mechanism which neutralized the ROS effect in humans, principle defensive agents against ROS is antioxidants and endogenous antioxidants (such as Catalase and superoxide dismutase (SOD), small proteins like thioredoxin, glutaredoxin, and molecules such as glutathione etc.) [3,4].But when the concentration of ROS increases beyond a certain limit it causes damage to DNA, Proteins, Lipids and carbohydrates, leads to oxidative stress. Various research proves that oxidative stress responsible for the development or enhancement of human diseases, such as ulcerative colitis (Ramakrishna et al.1997), nonulcer dyspepsia (Kumari et al. 2013), Parkinson's disease (Verma et al. 2015), Alzheimer's disease (Smith et al.2000), Atherosclerosis (Upston et al.2003), major depression(Bajpai et al.2014) alcohol induced liver disease (Arteel,2003),Cancer (Kinnula and Crapo,2004), diabetic nephropathy, end stage renal disease (Verma et al. 2014) Cardiovascular disease(Singh and Jialal, 2006), mild cognitive impairment (Guidi et al.,2006), aging(Hyunetal.2006) and neural disorders (Sas et al., 2007) [4-7].

Human body has a balanced system which maintained by DNA, Proteins, Carbohydrates and Lipids. When these biomolecules are damaged by ROS, causes disturbance in the metabolic state, growth and development of the cells of organism leads to serious disease, known as oxidative stress. Oxidative stress damages nitrogenous base, as well as strand breaks in DNA, such damage occurred by ROS generation. [E.g. superoxide radical (O_2^-), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2)] [8].Various radicals generated in our body such as hydroxyl radical, superoxide, and hydrogen peroxide etc., in which hydrogen peroxide is noted because it readily

permeates membrane, longer longevity (1min) and not compartmentalized in the cell [11]. Hydrogen peroxide produced during oxidative stresses, which are very reactive among the ROS, causes damage to Proteins, Nucleic acids, Carbohydrates and lipids leads to oxidative stress [9].

H_2O_2 react with biomolecules due to presence of transition metals such as Cu^{+}, and Fe^{++} (via Fenton reaction) to form the highly reactive hydroxyl radical which is the strongest oxidizing agent among other ROS, damage the DNA, proteins lipids and carbohydrates [9,20-22].

$Fe^{+2} + H_2O_2 \longrightarrow {}^{\cdot}OH + OH^{-} + Fe^{+3}$ (Fenton's reaction) [1-3]

1.1. Free radicals and reactive oxygen species

Free radicals are any atoms which have independent existence and contain one or more unpaired electrons in their outer valence shell, while Reactive oxygen species are all the free radicals that contain oxygen atoms and commonly found in biological system. ROS is two types, radicals [Superoxide (O_2^{-}), Hydroxyl ($^{\cdot}OH$) Peroxyl ($RO_2^{\cdot}$) Alkoxyl ($RO^{\cdot}$) Hydroperoxyl ($HO_2^{\cdot}$)] and Non-Radicals [Hydrogen peroxide(H_2O_2) Hypochlorous acid ($HOCl^{-}$), Ozone (O_3),Singlet oxygen($^1O_2$) and Peroxynitrite($ONOO^{-}$)] [8]

Reactive Species	Half life
Hydrogen peroxide, Hypohalous acids, Organic hydro peroxides	minutes
Peroxyl radicals, Nitric oxide	seconds
Peroxynitrite	milliseconds
Superoxide anion, Singlet oxygen, Alcoxyl radical's	microsecond
Hydroxyl radical	nanosecond

Table 1: Longevity of reactive species

1.2. Biological Pathways for Oxygen Reduction

Reactive oxygen species produced due to normal metabolism, radiation, inflammation, aging, chemical, drugs and so forth.

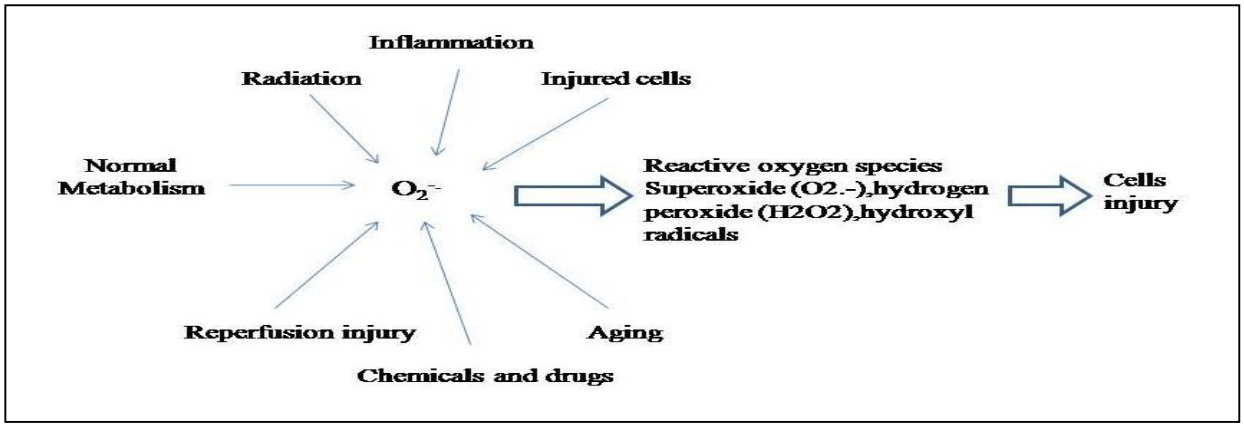

Figure 1

1.3. Types of ROS

1.3.1. Superoxide

Superoxide is not highly reactive, but it acts as a reducing agent because it converts ferric (Fe^{+++}) form of iron to ferrous (Fe^{++}) form. Superoxide present where they produced because they lack the ability to penetrate the lipid membranes. Superoxide spontaneously produced, especially in the electron rich aerobic environment of the inner mitochondrial membrane with respiratory chain. Formation of superoxide and hydrogen peroxide occurs endogenously by flavoenzymes, e.g. Xanthine oxidase generally activated the ischemia-reperfusion.

$Cu^{+2} / Fe^{+3} + O_2^{-} \longrightarrow Cu+ / Fe^{+2} + O_2$ [20]

1.3.2. Hydroxyl radical ($^{\cdot}OH$)

Hydroxyl radical is highly reactive than other ROS and damage biomolecules such DNA, Proteins, Carbohydrates and lipids. Hydroxyl radical formed from H_2O_2 via Fenton reaction in which H_2O_2 react with protein and other biomolecules which contains transition metals (Fe^{+2} or Cu^{+}).

$H_2O_2 + Cu^{+}/Fe^{+2} \longrightarrow OH^{-} + {}^{\cdot}OH + Cu^{+2}/Fe^{+3}$ (Fenton's reaction)

1.3.3. Hydrogen Peroxide (H_2O_2)

H_2O_2 is a pale-blue colour, covalent liquid which freely miscible with water. It can act as a mild oxidizing as well as mild reducing agent and react with proteins and other molecules which have transition metals, but not oxidize most biomolecules readily. Our body

makes H_2O_2 to fight against pathogens, it also provoke our immune system to function correctly. Neutrophils are leukocytes which produce hydrogen peroxide as the first line of defence against toxins, parasites, bacteria, viruses and yeast.

1.3.4. Hypochlorite (HOCl):

When H_2O_2 react with chlorine it forms the most reactive ROS, hypochlorite.

$$H^+ + Cl^- + H_2O_2 \longrightarrow HOCl + H_2O$$

1.3.5. Source of ROS

Exogenous source:

There are various source of production of ROS such as Radiation(UV light, x-rays, gamma rays), Chemicals that react to form peroxides (Ozone and singlet oxygen), Chemicals that promote superoxide formation(Quinones, nitro aromatics, bi pyrimidiulium herbicides), Chemicals that are metabolized to radicals (Poly halogenated alkanes, phenols, aminophenol),Chemicals that release iron (ferritin etc.) Above exogenous source of ROS formation occurs by the Fenton's and Haber's reaction.

Fenton's reaction: Molecular oxygen reduced to form superoxide which has the ability to form more and highly Reactive Oxygen Species and superoxide's dismutation forms hydrogen peroxide.

$$O^-_2 + O^-_2 + 2H \longrightarrow H_2O_2 + O_2$$

H_2O_2 react with transition metals such as Iron (Fe^{++}) /Copper (Cu^+) to form highly reactive hydroxyl radicals

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH \quad + OH^-$$

Haber-Weiss reaction:

H_2O_2 is more stable than superoxide, permeable to plasma membrane and plays two important roles in the body, either it is scavenged by enzymes Catalase /GSH(glutathione peroxidase), or it helps in the formation of ROS.

$$O_2 + H_2O_2 \longrightarrow O_2 + OH^- + {}^{\cdot}OH \text{ (Haber-Weiss reaction)}$$

H_2O_2 reacts with Halogen atoms such Cl^-, Br^-, and I^- and is utilized by Myeloperoxidase to form more reactive hypochloric acid / hyper chlorite. This is important for protein aggregation and fermentation.

$$H_2O_2 + Cl^- \longrightarrow HOCl + OH^-$$

Endogenous sources of ROS:

In the human body various enzymes such as monoamine oxidase, Lipoxygenase, Cyclooxygenase, NADPH oxidase, Cytochrome P450 Monooxygenase, Xanthine oxidoreductase and Nitric oxide synthase present which responsible for the generation of ROS under sub cellular.

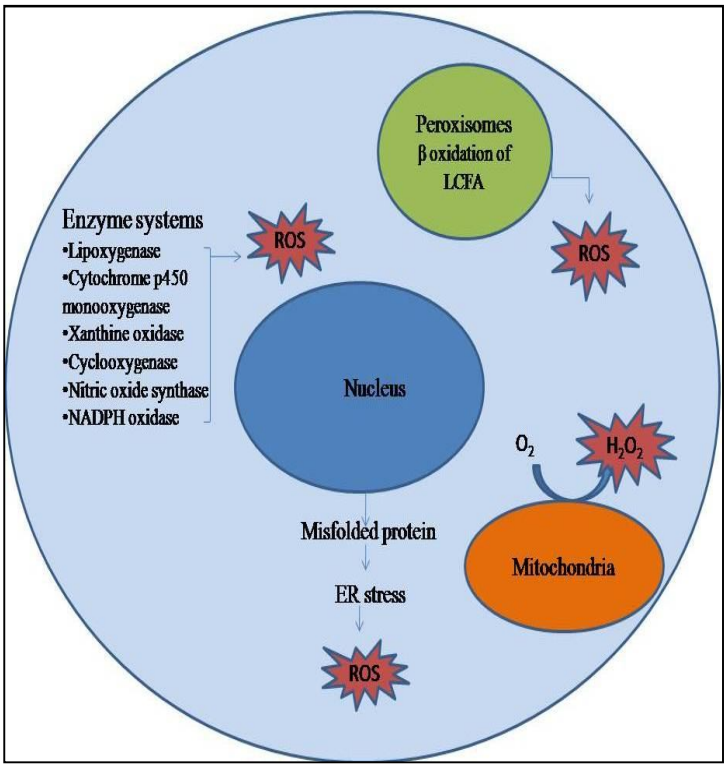

Figure 2

1.3.6. Monoamine Oxidase

Under normal physiological condition in inner mitochondrial membrane Electron Transport chain produces ATP and superoxide. While in outer mitochondrial membrane monoamine oxidase haem containing enzyme present which catalyzes oxidative deamination of amines and produces H_2O_2 in matrix and cytosol?

1.3.7. NADPH Oxidase /Respiratory Burst Oxidase

The stimulated production of ROS by phagocytic cells was originally called "the respiratory burst" due to the increased consumption of oxygen by these cells. This process is catalyzed by the action of NADPH oxidase, a multi component membrane bound enzyme complex, and is necessary for the bactericidal action of phagocytes. While several enzymes are recognized as being able to produce ROS moieties, NADPH oxidase is the most significant. NADPH oxidase activity is controlled by a complex regulatory system that involves the G-protein Rac (Figure 2).

$$2O_2 + NADP \longrightarrow \text{NADPH oxidase} \longrightarrow 2O_2^- + NADP^+ + H^+$$

1.3.8. Xanthine Oxidoreductase

This enzyme catalyzes hypoxanthine into Xanthine, then into uric acid. Xanthine Oxidoreductase (XOR) is present in the form of Xanthine Dehydrogenase (XD); these two forms of Xanthine are transformed. XD is transformed into XO, irreversibly by proteolysis and reversibly by oxidation of sulfhydryls, and produce large amount of H_2O_2 and O_2^-It is also found that XOR can transform nitrates into nitrites and NO. It also catalyzes the NO with O_2^- and form highly reactive Peroxynitrite.

1.3.9. Cytochrome P_{450} Oxidase

It is a haem-containing enzyme which present in mitochondria and participates in metabolism of cholesterol, hormones, steroids, catabolism of bile acids, arachidonic acid, eicosanoids, hydroxylation of vitaminD_3 and retinoid acid by catalyzing intramolecular transfer of oxygen. This enzyme transfers $2e^-$ one is bound to oxygen and the second is reduced to water.

1.3.10. Myeloperoxidase

Myeloperoxidase is haem containing enzyme, present in Neutrophils and eosinophils. It catalyzes the H_2O_2 with various substrates to form highly reactive hypochloric acids. When the concentration of ROS is low, it is beneficial and mediates the phagocytosis, apoptosis, detoxification reactions, executioner of precancerous cells and infections etc. ROS beneficially involved in signalling pathways to maintain cellular homeostasis in body. ROS regulates many metabolic as well as cellular processes such as proliferation, immunity, gene expression, migration and wound healing.

1.4. Site of ROS Generation

1.4.1. Production of ROS in Mitochondria

ROS are generated by mitochondria through the release of electrons from the electron transport chain where oxygen molecules reduced into superoxide (O_2^-), this superoxide changed into hydrogen peroxide with the help of superoxide dismutase (SOD). However hydrogen peroxide react with biomolecules which contains transition metals (Fe^{++},Cu^+) and produces hydroxyl radicals (Fenton's reaction) [32].

$$2O_2 + 2H_2O \longrightarrow O_2 + H_2O_2 + 2OH^-$$
$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

1.4.2. Production of ROS in endoplasmic reticulum

Cytochromes P_{450} complexes are used to detoxify the toxic hydrophobic chemical compounds of the body as a result, superoxide anions are formed. Enzyme Cytochrome P_{450} reductase is used to detoxify into hydrophilic compounds [36].

1.4.3. Peroxisomes generating hydrogen peroxide:

Peroxisomes containing enzymes such as glycolate oxidase, urate oxidase, fattyacyl CoA oxidase, d-amino acid oxidase and 1-α-hydroxyacid oxidase, are involved in generating H_2O_2. While Catalase enzyme involved in varieties of peroxidative reactions and convert hydrogen peroxide into water and oxygen molecules.

ROS Generation by Lysosomes: This system promotes 3 e^- reductions to oxygen and form highly reactive OH^-

Other source:

Auto oxidation of small molecules:

Small molecules like epinephrine, dopamine, flavins and hydroquinone's involve in direct production of O_2^-.

1.4.4. Viral infections associated with ROS

Many of the viral infections are associated with ROS generation, when intracellular and extracellular antioxidant level decreases.ROS and reactive nitrogen intermediates possess antimicrobial and antitumor activities, e.g.-Sendai and influenza viruses causes respiratory burst in phagocytic cells and elevates the ROS/RNS concentration of the cell.HIV increases oxidative stress by stimulating transcription factor NFκB (Nuclear Factor κB),cytokines and TNF-α, which may result in release of H_2O_2 from T-Cells. While Hepatitis virus directly affects the host genome and results in the production of ROS. It is characterized by increased cell proliferation, which ultimately becomes cancer [26].

1.5. ANTIOXIDANT

Term antioxidant widely used but it is difficult to define clearly. Food scientist use antioxidant which inhibit lipid peroxidation. Polymer scientist use antioxidant to control polymerization in rubber, plastic and paint manufacture. Antioxidant are those substances

which present at low concentration compared with oxidizable substrate (every type of molecule found in vivo), significantly delays or prevents oxidation of that substrate. [1, 8, 11, 12]

- Classification of antioxidants: Antioxidants are classified into 3 categories, as described by Gutteridge and Halliwell [13].
- Primary antioxidants: It is involved in the prevention of oxidants formation.
- Secondary antioxidants: It exhibits scavenger of ROS.
- Tertiary antioxidants: It repairs the oxidized molecules through sources like dietary or consecutive antioxidants.

1.6. Types of Antioxidant

Antioxidants may be enzymatic and non-enzymatic in nature in which enzymatic system directly or indirectly help in defence against the ROS. (E.g.-Superoxide dismutase (SODs remove superoxide by accelerating its conversion into Hydrogen peroxide. SOD enzyme contains manganese (MnSOD), copper and Zinc (CuZnSOD) at its active site in mitochondria and cytosol respectively), Catalase (This enzyme converts H_2O_2 to water and oxygen.), glutathione peroxidase (In human cells GSHPX is most important H_2O_2 removing enzyme it requires selenium for their action. GSHPX enzymes remove H_2O_2 by using it to oxidize reduced glutathione to oxidized glutathione), glutathione reductase (it is a flavoprotein enzyme regenerates reduced glutathione from oxidized glutathione), and thioredoxin) [].While non-enzymatic antioxidant act as scavenger of ROS and RNS.eg Vitamin E (Inhibit lipid per oxidation by scavenging Peroxyl radical intermediates), Vitamin C, and Vitamin A, glutathione, uric acid and melatonin (It react with ROS and form disulfide) [23-26].

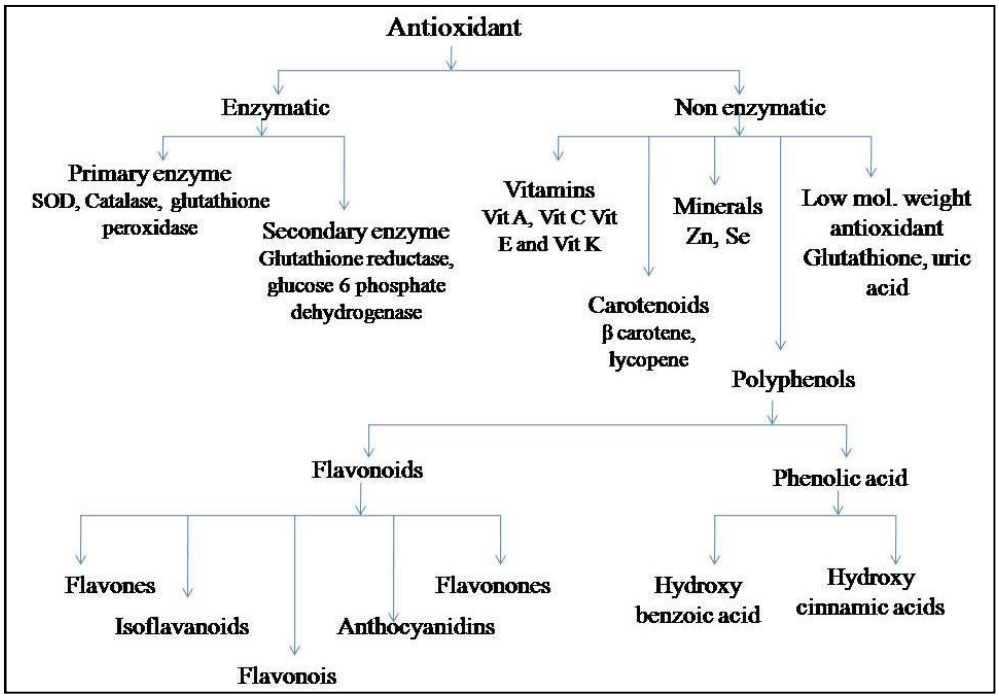

Figure 3

Note: Some amino acids also have antioxidant property such as Cysteine, Methionine, Taurine (it derived from Methionine and Cysteine metabolism in vivo) [Atmaca, 2004] [2, 15].

Types of Antioxidants on the basis of solubility:

Hydrophilic antioxidants: Such type of Antioxidants reacts with oxidants in the cell cytoplasm and the blood plasma. e.g. Ascorbic acid, Glutathione, Uric acid and so forth.

Hydrophobic antioxidants:

Such type antioxidant protects cell membranes from lipid per oxidation.eg. Carotenes, α-Tocopherols and Ubiquinols. These compounds may be synthesized in the body or obtained from the diet.

ROS Scavengers	ROS Protective Enzymes	Sequestration of Transition Metal Ions
Glutathione	Superoxide dismutase(SOD)	Transferrin
Ascorbic acid(Vitamin C)	Glutathione peroxidase	Metallothionein
Uric acid	Catalase	Ferritin
Albumin	Glutathione reductase	Ceruloplasmin

Table 2: Important antioxidants & endogenous antioxidant enzymes
ANTIOXIDANT DEFENSIVE AGENTS

The cell damage through free radical mediated reactions can be protected by enzymatic and non-enzymatic defence mechanisms. Antioxidant system contains endogenous antioxidants and exogenous antioxidant (dietary sources).

1.7. Endogenous antioxidants

It can be categorized into primary and secondary antioxidants. Primary antioxidant enzymes such as SOD, Catalase, and Glutathione peroxidase inactivate the ROS into intermediates. Besides the antioxidant enzymes, primary antioxidants also water soluble (Ascorbate, glutathione, and uric acid etc) and lipids soluble (Tocopherols, ubiquinols and Carotenoids, etc) in nature. Secondary antioxidant enzymes are Glutathione reductase, glutathione-S-transferase, Glucose-6-Phosphate dehydrogenase, and ubiquinone helps to detoxify ROS by lowering the peroxides level and continuously supplying the NADPH and glutathione for primary antioxidant enzymes to maintain their proper functioning. Copper, iron, manganese, zinc, and selenium enhance the antioxidant enzyme activities.
Exogenous antioxidants: These are mainly derived from food and other dietary sources. Several herbs, spices, vitamins, foods, and vegetables etc exhibits antioxidant activities.

1.8. Oxidative Stress

According to Sies oxidative stress is "a disturbance in the prooxidant and antioxidant balance in favour of prooxidant leading to serious damage of biomolecules". Term oxidative stress refers to the imbalance between the production of reactive species and antioxidant defence. Oxidative stress focuses the attention of worldwide researchers for its deleterious effects on biomolecules such as DNA, Proteins, Lipids and carbohydrates, etc in the human body [27-29]

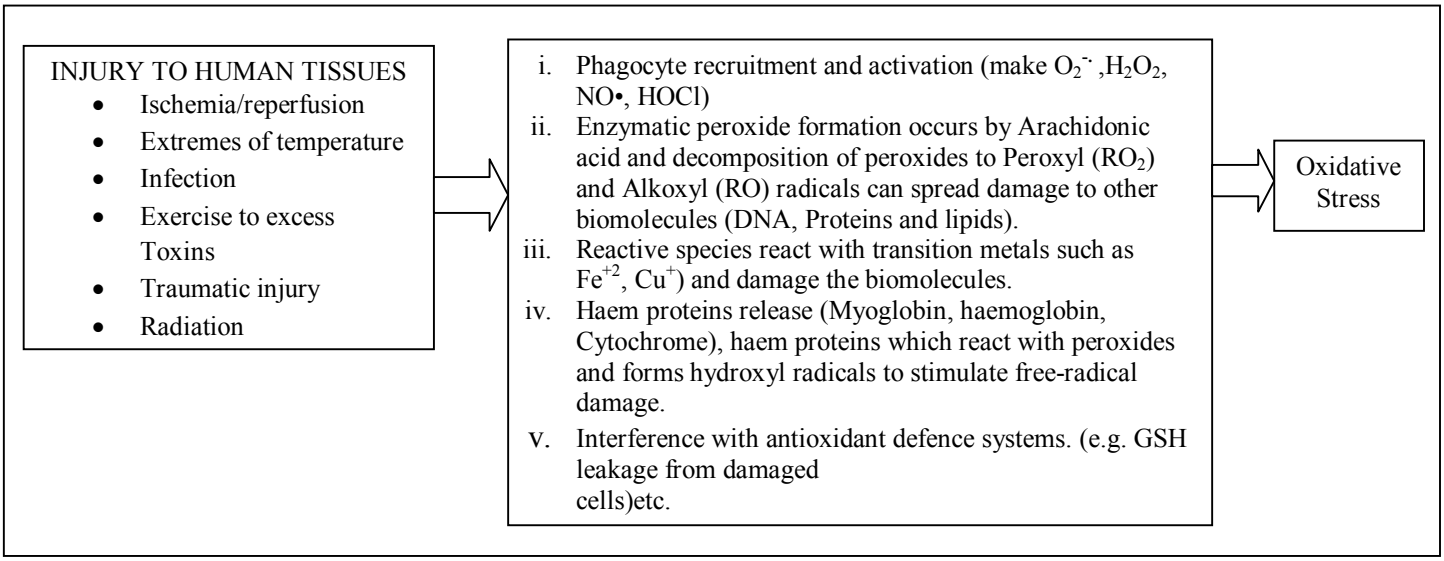

Figure 4

1.9. Oxidative Stress and Disease

Oxidative stress is imbalance between production of reactive species and antioxidant defence leading to several diseases in humans. Free radicals and other reactive species implicated in the pathology causes over 100 human diseases, such as atherosclerosis, cancer and AIDS nonulcer dyspepsia (Kumari et al. 2013), Parkinson's disease (Verma et al. 2015), Alzheimer's disease (Smith et al.2000), Atherosclerosis (Upston et al.2003), major depression (Bajpai et al.2014) , diabetic nephropathy, end stage renal disease (Verma et al. 2014) Cardiovascular disease(Singh and Jialal, 2006) etc. [4-7].

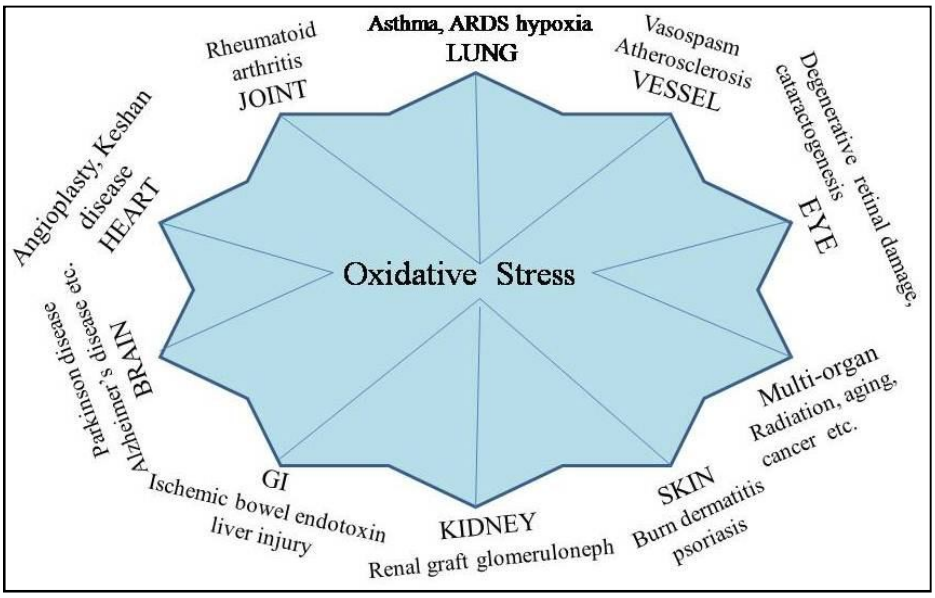

Figure 5

2. Conclusion

Various kinds of stresses such as the oxidative stress, heat stress, and other denaturing stresses perturb the structure of Protein, Carbohydrates, Lipids and DNA molecules.ROS causes the oxidative stress, leads to several human diseases such as neurodegenerative disease, immune disease, arteriosclerosis, rheumatoid arthritis, diabetes as well as cancer.

In human body various reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals are generated in which hydroxyl radical is highly reactive and prone to damage various biomolecules.

Due to oxidative process H_2O_2 can react with transition metals such as iron, copper etc to generate the highly reactive hydroxyl radical, which can oxidize a variety of biological molecules. H_2O_2 can also react with Hb and other haem proteins. Hbs alone has a higher oxidation state of iron, Fe^{+4}, which can initiate membrane lipid per oxidation and oxidize other macromolecules. Haem is a fundamental molecule for living organisms, as the cofactor for several proteins and enzymes involved in cellular processes (transport of gases, redox reactions and electron transport).ROS are highly reactive and damage various biomolecules such as Proteins, Carbohydrates, Lipids and DNA molecules. Those substances have the capacity to scavenge the ROS and protect the biomolecules from injury is known as Antioxidants. Various researches prove that several enzymes (SOD, Catalase etc), Vitamins (Vit A, Vit C and Vit E) and amino acids (Cysteine and Methionine etc.) have antioxidant property.

3. References

i. Barry Halliwell "Free Radicals and other reactive species in Disease" ENCYCLOPEDIA OF LIFE SCIENCES / & 2001 Nature Publishing Group.

ii. Anand Kumar Keshari, Humaira farooqi "Evaluation of the effect of hydrogen peroxide(H_2O_2) on haemoglobin and the protective effect of glycine'' International journal of science & Tecnhnoledge,vol 2, issue2,2014.

iii. Klaus Apel, Heribert Hirt "Reactive oxygen species: Metabolism, Oxidative Stress, and Signal Transduction" Annu. Rev. Plant Biol. 2004. 55:373–99

iv. By Paul Held, Laboratory Manager, Applications Dept.,BioTek Instruments, Inc. "An Introduction to Reactive Oxygen Species & Measurement of ROS in Cells".

v. Akhilesh Kumar Verma, Janak Raj, Vivek Sharma, Tej Bali Singh, Shalabh Srivastava, and Ragini Srivastava, "Plasma Prolidase Activity and Oxidative Stress in Patients with Parkinson's Disease," Parkinson's Disease, Article ID 598028, in press.

vi. Bajpai, A., Verma, A. K., Srivastava, M., & Srivastava, R. (2014). Oxidative Stress and Major Depression. Journal of Clinical and Diagnostic Research : JCDR, 8(12),

vii. Shweta Kumari, Akhilesh Kumar Verma, Sumit Rungta, Rahul Mitra, Ragini Srivastava, and Narender Kumar, "Serum Prolidase Activity, Oxidant and Antioxidant Status in Nonulcer Dyspepsia and Healthy Volunteers,"ISRN Biochemistry, vol. 2013, Article ID 182601, 6 pages, 2013.

viii. Akhilesh Kumar Verma, Subhash Chandra, Rana Gopal Singh, Tej Bali Singh, Shalabh Srivastava, and Ragini Srivastava, "Serum Prolidase Activity and Oxidative Stress in Diabetic Nephropathy and End Stage Renal Disease: A Correlative Study with Glucose and Creatinine," Biochemistry Research International, vol. 2014, Article ID 291458, 7 pages, 2014.

ix. Shafaq Noori "An Overview of Oxidative Stress and Antioxidant Defensive System" Open Access Scientific Reports volume 1, 2012.

x. Anu Rahal, Amit Kumar, Vivek Singh, Brijesh Yadav,Ruchi Tiwari, Sandip Chakra borty, and Kuldeep Dhama "Oxidative Stress, Prooxidant, and Antioxidants: The Interplay" BioMed Research International Volume 2014.

xi. David Landsborough Thomson, The effect of hydrogen peroxide on the permeability of the cell,1927.
xii. Shakir Ali, Humaira Farooqi, Ram Prasad , Mohammad Naime , Indusmita Routray , Savita Yadav , Faizan Ahmad "Boron stabilizes peroxide mediated changes in the structure of heme proteins" International Journal of Biological Macromolecules 47 (2010) 109–115
xiii. Marian Valko, Dieter Leibfritz, Jan Moncol , Mark T.D. Cronin ,Milan Mazur , Joshua Telser, "Free radicals and antioxidants in normal physiological functions and human disease" The International Journal of Biochemistry & Cell Biology 39 (2007) 44–84.
xiv. Blokhina O,Virolainen E,Fagerstedt KV(2003) " Antioxidant, oxidative damage & oxygen deprivation stress": a review Ann Bot 91: 177-194.
xv. Halliwell B, Gutteridge JMC "Free Radicals in Biology and Medicine "Clarendon Press, Oxford, U 1989.
xvi. Gulizar Atmaca "Antioxidant effect of sulphur containing amino acids" Yonsei Medical Journal,2004, vol 45 pp 776-778
xvii. Rahmat Ali Khan, Muhammad Rashid Khan, Sumaira Sahreen and Mushtaq Ahmed "Evaluation of Phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill" Chemistry Central Journal 2012, 6:12
xviii. Djordje Melanie Milan Popover and Jegor Miladinović "Phenolic Content and Antioxidant Properties of Soybean (Glycine max (L.) Merr.)" Seeds Molecules, 2007, 12, 576-581
xix. Hui-Chun Wu, Hua-Ming Chen, Chyuan-Yuan Shiau, "Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus)" Food Research International 36 (2003) 949–957
xx. Klaus Apel ,Heribert Hirt "Reactive oxygen species : Metabolism, Oxidative Stress, and Signal Transduction" Annu. Rev. Plant Biol. 2004. 55:373–99.
xxi. Barry Halliwell, Marie Veronique Clementb, Lee Hua Longa a " Hydrogen peroxide in the human body" Minireview.
xxii. Stedman ER oliver CN(1991) "Metal catalysed oxidation of protein. Physiological consequence. J Bio chem. 266: 2005-2008.
xxiii. Nam Hoon Kim and Jung Hoon Kang, "Oxidative Damage of DNA Induced by the Cytochrome c and Hydrogen Peroxide System, Journal of Biochemistry and Molecular Biology"July 2006, pp. 452-456.
xxiv. Marcin Kruszewski1,and Teresa Iwaneñko, Labile iron pool correlates with iron content in the nucleus and the formation of oxidative DNA damage in mouse lymphoma L5178Y cell lines, Acta Biochimica Polonica, Vol. 50,2003 211–215.
xxv. Dejia Li!, Xu Zhang!, Yue Loni, Ximeng Sun "Inactivation of Hemoglobin by Hydrogen Peroxide and Protection by a Reductant Substrate" Life Science Journal ,2006.
xxvi. Amit Kumar Mandal a, Murali Woodi b, Varun Sood a, Patnam Rajagopalan Krishnaswamy b, Anjali Rao c, Sudarshan Ballal b, Padmanabhan Balaram, Quantitation and characterization of glutathionyl haemoglobin as an oxidative stress marker in chronic renal failure by mass spectrometry, Clinical Biochemistry 40 (2007) 986–994.
xxvii. Yasir Hasan Siddique, Tanveer Beg and Mohammad Afzal "Protective effect of ascorbic acid against oxidative damage induced by hydrogen peroxide in cultured human peripheral blood lymphocytes" Indian Journal of Clinical Biochemistry, 2009.
xxviii. Elisa Cabiscol Jordi Tamarit Joaquim Ros "Oxidative stress in bacteria and protein damage by reactive oxygen species" INTERNATL MICROBIOL Vol. 3, 2000
xxix. Paul K. Witting, D. J. Douglas, and A. Grant Mauk, Reaction of Human Myoglobin and H_2O_2. The journal of biological chemistry Vol. 275, 2000
xxx. Manat Chaijan, Lipid and myoglobin oxidations in muscle foods, Songklanakarin J. Sci. Technol.30 (1), 47-53, Jan. - Feb. 2000
xxxi. Toshitaka Matsui, Shin-ichi Ozaki, Elaine Liong George N. Phillips, Jr. and Yoshihito Watanabe, Effects of the Location of Distal Histidine in the Reaction of Myoglobin With Hydrogen Peroxide, The journal of biological chemistry Vol. 274, pp. 2838–2844, 1999.
xxxii. S. Seal, S.C. Kuiry, B. Heinmen, Effect of glycine and hydrogen peroxide on Chemical-mechanical planarization of copper, Thin Solid Films,2003, 243– 251.
xxxiii. Jian-Ming Li, Qian Cai, Hong Zhou, Guang-Xia Xiao, Effects of hydrogen peroxide on mitochondrial gene expression of intestinal epithelial cells, World J Gastroenterol 2002;8(6):1117- 1122.
xxxiv. Akio Tomoda,Kazu Sugimoto, Masahiko Suhara,T Masazumi Takeshitat and Yoshimasa Yoneyama, Haemichrome Formation from Haemoglobin Subunits by Hydrogen Peroxide, Biochem. J,1978, 329-335.
xxxv. Tsuyoshi Egawa, Hideo Shimada and Yuzuru Ishimura, Formation of Compound I in the Reaction of Native Myoglobins with Hydrogen Peroxide.
xxxvi. Martin Newcomb, David Aebisher, Runnan Shen, R. Esala P. Chandrasena,Paul F. Hollenberg,and Minor J. Coon, Kinetic Isotope Effects Implicate Two Electrophilic Oxidants in Cytochrome P450-Catalyzed Hydroxylations Ortiz de Montellano, P. R., Ed. Cytochrome P450 Structure, Mechanism, and Biochemistry, 2nd ed.; Plenum: New York, 1995