ABSTRACT:

Free radicals are the unstable electron-deficient species that reacts with different molecules to gain stability and to eliminate their unpaired condition. Antioxidant molecules neutralizes the free radicals by donating their electrons and inhibits the unwanted oxidative reactions in biological system. The imbalance between antioxidants and free radicals generated oxidative stress which leads to severe impairment of the biological systems. The purpose of the present review is to highlight the beneficial role of naturally occurring antioxidant systems in minimizing the damage and maintaining the homeostasis in the biological system. Enzymatic and non-enzymatic antioxidants are the major classes of natural antioxidants which executes diverse functions in the biological system to provide defense against the destructive accumulating effects of ROS/RNS. Superoxide dismutase (SOD) are responsible for providing first line of defense to the biological system, by converting the superoxide anion radical ($O_2^-$) into hydrogen peroxide ($H_2O_2$) which eventually converted into water and oxygen. Non-enzymatic antioxidants either endogenous or exogenous provides numerous crucial mechanisms to quench the ROS/RNS in the biological system. Endogenous antioxidants inhibits lipid peroxidation in the cellular membranes while the exogenous antioxidants acts as chain breakers and terminates the oxidative chain reaction. It is significant to sustain the level of exogenous antioxidants in the body through diet so that the normal biological processes can be maintained at optimal levels.

Keywords: Antioxidants; free radicals; oxidative stress; homeostasis; protection
INTRODUCTION

In the biological system, normal metabolic activities and ATP (energy) production relies on oxygen because it acts as the final electron acceptor in cellular respiration (Stanley and Chandler, 2002). Difficulties occur when the energy production and electron flow become disengaged which leads to generation of free radicals (FR) in the biological system (Nohl et al., 2005). FR are unstable and highly reactive molecules, produced during oxidative/cellular metabolic processes and due to the presence of unpaired electrons, FR can easily react with other molecules to acquire stability (Nasri, 2016). FR have a half-life of about $10^{-10}$ seconds and their reactivity depends upon their extent of stability in a particular system (Suma, 2014). FR are the derivative of oxygen and nitrogen, termed as reactive oxygen and reactive nitrogen specie, ROS and RNS respectively. ROS/RNS have significant roles in cellular apoptosis (programmed cell death), cellular signaling pathways, ion transportation and gene expression (Franco et al., 2009). FR formed due to the exogenous sources includes the contaminants, chemicals, ionizing radiation, toxins while those which are produced from the endogenous sources includes the proteins, enzymes (Xanthine Oxidase and Monoamine Oxidase) and other organic molecules (Valko et al., 2006; Dhiman et al., 2017). Excessive level of ROS attacks and damages the proteins, nucleic acids, side chains of different amino acids, unsaturated fatty acids, DNA and RNA, thereby generating oxidative stress in the body (Baeza et al., 2010; Holmgren and Lu, 2010; Lu et al., 2010). RNS produced in insignificant amounts during usual cellular processes; ATP production for cellular energy, neurotransmission and cell signaling, blood pressure modulation, phagocytosis and regulation of cell growth (Santos-Sanchez et al., 2019). Oxidative stress is a complex yet harmful response evoked by ROS/RNS in the body of living organism (Colin-Gonzalez et al., 2015), which is generated when ROS/RNS production go beyond the defense capacity of the cellular antioxidants (Limon-Pacheco and Gonsebatt, 2009). Oxidative stress existed at tissue, cellular, molecular and even at genetic levels of all living organisms and characterized by the accumulation of severe deleterious impacts on the cells and tissues which increases the risk of diseases and death (Rahal et al., 2014). In response to various stimuli, the body immunity is suppressed due to the presence of oxidative stress, inflammatory reactions, incidence of apoptosis (programmed cell death) and various devastating reactions (Cooksey et al., 2004, Galanis et al., 2009). Oxidative stress ultimately intensifies the risk for various deadly diseases which includes cancer, autism, cardiovascular disease, rheumatoid arthritis, diabetes and many other (Table 1) (Valko et al., 2007; Piwkowska et al., 2011).
Table 1: Positive Correlation of Oxidative Stress with Different Diseases

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Etiology</th>
<th>Organs</th>
<th>Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Reactive oxygen intermediates</td>
<td>Eyes</td>
<td>Macular degeneration</td>
<td>(Beatty et al., 2000)</td>
</tr>
<tr>
<td>2.</td>
<td>ROS</td>
<td>Multi-organ</td>
<td>Diabetes</td>
<td>(Maritim et al., 2003)</td>
</tr>
<tr>
<td>3.</td>
<td>Reactive C-protein</td>
<td>Multi-organ</td>
<td>Chronic fatigue</td>
<td>(Fulle et al., 2007)</td>
</tr>
<tr>
<td>4.</td>
<td>NADPH (Reduced) oxidase system</td>
<td>Blood vessels</td>
<td>Atherosclerosis</td>
<td>(Singh and Jialal, 2006)</td>
</tr>
<tr>
<td>5.</td>
<td>ROS</td>
<td>Brain</td>
<td>Neurodegenerative diseases</td>
<td>(Uttara et al., 2009)</td>
</tr>
<tr>
<td>6.</td>
<td>ROS, H$_2$O$_2$</td>
<td>Lungs</td>
<td>Asthma</td>
<td>(Dozor, 2010)</td>
</tr>
<tr>
<td>7.</td>
<td>ROS</td>
<td>Joints</td>
<td>Rheumatoid and osteoarthritis</td>
<td>(Blackburn et al., 2011)</td>
</tr>
<tr>
<td>8.</td>
<td>Glutathione transferase kappa</td>
<td>Kidney</td>
<td>Nephritis</td>
<td>(Ziskoven et al., 2010)</td>
</tr>
<tr>
<td>9.</td>
<td>Lipid peroxidation (LPO) and DNA damage</td>
<td>Skin</td>
<td>Melanoma</td>
<td>(Sander et al., 2003)</td>
</tr>
<tr>
<td>10.</td>
<td>ROS</td>
<td>Heart</td>
<td>Myocardial infarction</td>
<td>(Filippo et al., 2006)</td>
</tr>
</tbody>
</table>

In the biological system, oxidative stress irreplaceably activates the phagocytic system and causes destruction of various proteins and enzymes by reducing, carboxylating, peroxidating or hydroxylating the side chains of amino acids (Grimsrud et al., 2008; Forman et al., 2014).

Antioxidant molecules are capable of neutralizing FR by contributing their electron to eradicate the unpaired condition of FR and slow down the oxidation process (Rahman, 2007; Mathew et al., 2011). In biochemistry, antioxidants are defined as, the substances either enzymatic or non-enzymatic organic molecules, capable of neutralizing the deleterious effects of FR in biological system (Suma, 2014). Antioxidants commonly act as reducing agents because they are being oxidized in process to halt the oxidative chain reactions in body by eliminating the FR (Kabel, 2014). Low levels of antioxidants cause massive damage to the body; often associated with heart diseases and cancer but abnormal cell division is reduced when antioxidant level becomes normal.
The global natural antioxidants market is expected to grow at a rate of 8.4% annually from 2.22 Billion to 4.14 Billion USD by 2022 (Prakash et al., 2020). In vitro non-enzymatic antioxidant activities (DPPH, ABTS, Iron Reducing Power) are used as initial screening analysis to determine the antioxidant ability of natural as well as synthetic compounds (Tajammal et al., 2017; Yasmin et al., 2020).

In biological system, antioxidants reduce the level of cellular FR in two ways; either by lowering the expressions of those enzymes which are involve in the formation of FR (NAD(P)H oxidase and xanthine oxidase) or by uplifting the expressions of enzymatic antioxidants; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx). The purpose of the present review is to highlight the beneficial role of naturally present antioxidants in minimizing the damage and maintaining the homeostasis of the biological system. Synthetic antioxidants are not being discussed as part of this review.

1. Classification of Antioxidants

Antioxidants are classified based on their existence as natural and synthetic antioxidants. Natural antioxidants are present naturally in human body (endogenous antioxidants) and in plants (exogenous antioxidants). The natural antioxidants found in humans can be further classified on the basis of their nature and activity as enzymatic and non-enzymatic antioxidants (Fig. 1).

![Fig. 1: Schematic Diagram for the Natural Antioxidants and their Major Classes](image-url)
1.1. Enzymatic Antioxidants and their Role in Biological System

Body protects itself from the harmful effects of FR by using enzymatic antioxidant system as first line of defense and these antioxidant enzymes are essential in reducing the levels of lipid hydro-peroxides and \( H_2O_2 \) (major form of ROS). (Masella et al., 2005; Lu et al., 2010). Most abundant group of antioxidants in the living organisms consist of enzymes from the SOD family, which converts superoxide anions to hydrogen peroxide \( (O_2^\cdot\rightarrow H_2O_2) \) by reduction which in turn decomposes either by GSHPx or by CAT (Rahal et al., 2014). These enzymes function synergistically to neutralize the FR as shown in Fig. 2 (Bonner and Arbiser, 2014).

\[
O_2^\cdot \xrightarrow{(SOD)} H_2O_2 \xrightarrow{(GSHPx)} \xrightarrow{(CAT)} H_2O + \frac{1}{2} O_2
\]

Fig. 2: General Mechanism of Action of Enzymatic Antioxidants (Rahal et al., 2014)

1.1.1 Superoxide Dismutase

Superoxide Dismutase (SOD) an enzymatic antioxidant present in the biological system which reduces oxidative damage and repairs the cells by eliminating superoxide anionic radicals \( (O_2^\cdot) \) (Limon-Pacheco and Gonsebatt, 2009, Abskharon et al., 2010). It plays vital role in defense of cellular constituents of biological system from toxic side effects of ROS (Johnson and Giulivi, 2005) by converting \( O_2^\cdot \) to \( H_2O_2 \) (Zelko et al., 2002). Previously it was established that SOD has potential role in suppressing the cell death in ovarian follicles of cultured rats, and transgenic mice by inhibiting NO to be converted into peroxynitrate, which induces apoptosis (Keller et al., 1998). Mostly aerobic cells and extracellular fluids contain SOD with different metal ions which can be Zn, Cu or Mn. In humans, Cu/Zn-SOD form is present in cytosol of cell while Mn-SOD is present in cellular mitochondria (Kabel, 2014). Cu/Zn-SOD form has two similar subunits (32 kDa) and each subunit have binuclear metal clusters of Cu and Zn ions in active sites of enzyme, that provides first line of defense to the system by catalyzing the dismutation reaction of superoxide radical. While Mn-SOD enzyme, is a 96 kDa homotetramer which has one Mn atom in each of its subunit, and it cycles between Mn(III) to Mn(II) form throughout the two-step dismutation process (Rahman, 2007).

1.1.2 Catalase

A well-known catalase named \( H_2O_2 \) oxido-reductase have four polypeptide chains in its structure and these chains contain more than 500 amino acids each and four porphyrin haem groups. Peroxisomes of all mammalian cells contain this enzyme except erythrocytes (Kabel, 2014). The optimum pH for CAT in humans is
approximately neutral while in other organism it varies from 4 to 11 depending on the species (Bahorun et al., 2006). In mammalian cells, H$_2$O$_2$ is produced by various oxidases and acts as a substrate in many biological reactions that produce highly reactive hydroxyl radicals but CAT enhances the body's antioxidant defense mechanism by inhibiting H$_2$O$_2$ accumulation (Ho et al., 2004). Two-stage break-down mechanism is used for H$_2$O$_2$ in which the haem iron at active site of the CAT enzyme oxidizes and reduces alternatively. In the first step, one H$_2$O$_2$ molecule oxidized the haem moiety to an oxyferryl specie while in the second step, second H$_2$O$_2$ molecule regenerates the enzyme which acts as reductant and NADPH act as a cofactor. Millions of H$_2$O$_2$ molecules decomposed by CAT to water and oxygen and it follows the fashion of a first-order reaction and we know that 1st order reaction rate is dependent on the concentration so here rate is dependent on H$_2$O$_2$ concentration (Rahman, 2007). Catalase deficient patients are normal phenotypically but due to excessive tissue damage caused by hydrogen peroxide, tendency to develop progressive oral gangrene is increased (Kabel, 2014).

### 1.1.3 Glutathione peroxidase

Glutathione peroxidases (GSHPx) are present in cytoplasm as well as in mitochondrial matrix at mM concentrations to remove hydrogen peroxide by cycling between reduced (GSH) and oxidized form of Glutathione (GSSG). GSHPx donates two electrons to reduce peroxides by forming selenoles (Se-OH), and can also reduce fatty acid hydro-peroxides (Limon-Pacheco and Gonsebatt, 2009). There are two forms of Glutathione peroxidase; selenium dependent GSHPx and selenium-independent glutathione-S-transferase (GST). The seleno-protein GSHPx enzyme removes H$_2$O$_2$ and oxidize GSH into GSSG (Pham-Huy et al., 2008). Furthermore, in humans, four isoforms of Se-dependent glutathione peroxidases are present which removes the peroxides and works in association with GSH to catalyze the conversion of organic peroxides to alcohol or water. A recent study indicates that the level of enzymes; GSHPx, GST and GSH increases by the administration of ethanolic extracts of A. maurorum in STZ-NA induced diabetic rats due to the presence of flavonoids, it also reduces the high MDA level and oxidative stress; common feature of diabetes (Sheweita et al., 2016).

### 1.1.4 Thioredoxin System

The thioredoxin system is a thiol-specific antioxidant system of oxido-reductase enzymes which consists of NADPH, thioredoxin (Trx) and thioredoxin reductase (TrxR) (Miranda-Vizuete et al., 2000). Two neighboring cysteine units are present in the active site of Trx which cycle between an active di-thiol form (reduced) to an oxidized disulfide form (Arner et al., 1999). The TrxR carried out the reduction of disulfide in the active sites of Trx with the help of NADPH and the reduced form of Trx is
a common reductant of disulfides residues in different proteins (Fig. 3) (Nordberg and Arnar, 2005).

TrxR reduces the disulfide in Trx by directly consuming the NADPH (Pham-Huy et al. 2008). Trx acts as an effective reducing agent for ROS/RNS in its active state (Kabel, 2014). Antioxidant systems are differentially expressed at the transcriptional level in adult organs and embryonic stages in mice while the Trx antioxidant system is activated in conditions of high oxidative stress (Limon-Pacheco and Gonsebatt, 2009, Holmgren and Lu, 2010).

Until now, three variants of Trx in human are identified which are encoded by different genes. Trx-1 is a 12-kDa variant of Trx and maximally studied gene which is highly expressed in kidneys, lungs and minute amount being present in heart, brain, and testis (Chen et al., 2008). The Trx-2 variant of Trx has 60 amino-acid long peptide chain which signals in mitochondrial translocation and maximal expression of Trx-2 identified in kidneys and heart while minimal levels exist in lungs. SpTrx is a third Trx variant highly expressed in spermatozoa (Bindoli et al., 2009). A conserved active site of -Cys-Gly-Pro-Cys- is present in all variants Trx (Trx-1, Trx-2, and SpTrx) in humans and all other organisms which is important for its antioxidant function as disulfide oxido-reductase for proteins. (Oslowski et al., 2012).

1.2 Non-Enzymatic Antioxidants and their role in Biological System

Non-Enzymatic antioxidants consist of endogenous as well as exogenous antioxidants. The endogenous antioxidants are produced in the human body during metabolic reactions while exogenous antioxidants cannot be produced in the body and must be provided through foods or supplements. These exogenous antioxidants supplementation are important to eradicate the unnecessary internal ROS, while improve the disease resistance capability (Jamshidi-Kia et al., 2020). Microalgae are found to be abundant source of natural exogenous antioxidants, possibly due to presence of phenolic compounds, vitamins, carotenoids and fatty acids (Qiu et al., 2020). These antioxidants reduces the generation of free radicals specially ROS (Table 2).
Table 2: Non-Enzymatic Antioxidants and their Location

<table>
<thead>
<tr>
<th>Non-Enzymatic Antioxidants</th>
<th>Category</th>
<th>Location/ Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Lipoic Acid</td>
<td>Endogenous</td>
<td>Cellular membranes, Cytosol</td>
</tr>
<tr>
<td>Coenzyme Q 10</td>
<td>Endogenous</td>
<td>Cellular membranes</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Endogenous</td>
<td>Plasma</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Endogenous</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Endogenous</td>
<td>Cell's mitochondria</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Exogenous</td>
<td>Fruits, Vegetables</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Exogenous</td>
<td>Wheat germ, egg yolks and nuts.</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Exogenous</td>
<td>Plants, fruits</td>
</tr>
</tbody>
</table>

1.2.1 α-Lipoic Acid

α-Lipoic Acid (α-LA) is an important endogenous antioxidant which is majorly dispersed in cell membrane and cytosol of both eukaryotic and prokaryotic organisms (Rahman, 2007). Indirectly it maintains the level of antioxidants in cells by increasing the formation of endogenous antioxidants such as GSH (Rajakumar et al., 2016). α-LA and its reduced form; dihydrolipoic acid (DHLA) both are potential antioxidants and involved in scavenging the FR, repairing protein impairment in cytosol of cells which is caused by the oxidative stress, regenerating the antioxidants and chelating the metal ions. DHLA is more potent antioxidant than α-LA and can act synergistically with other antioxidants (Rahman, 2007). α-LA has an significant role in improving insulin sensitivity, mitochondrial oxidative phosphorylation expression, α-oxidation capacity, intracellular ATP production, and lowering the endoplasmic reticulum stress (Lei et al., 2016). Low pH enhances lipids oxidation, for instance, oxidation of linoleic acid and polyunsaturated fatty acids is increased at low pH and occurred rapidly (Goyal and Kaur, 2019). A study revealed that DHLA has a protective effect on the person suffering from Alzheimer's disease by decrease the accumulation of excess iron in the body which in turns reduces the production of ROS (Mudd et al., 2016).

1.2.2 Coenzyme Q10

An endogenous antioxidant; synthesized in intercellular region by using tyrosine as fundamental building blocks (Pahari et al., 2016). It exists biochemically in redox form in all the biological tissues, ubiquinol (reduced) and ubiquinone (oxidized) form which is useful biomarker of oxidative stress. In the reduced form, it holds electrons loosely; it can give up easily to neutralize the FR. Reduced form of CoQ10 displays strongest antioxidant...
action (Maladkar et al., 2016) by inhibiting lipid peroxyl radicals production and even neutralize the already present FR (Carocho and Ferreira, 2013). It protects membrane phospholipids from damage caused by peroxides and FR-induced oxidation (Sarmiento et al., 2016) and also involved in the prevention of liver fibrosis by lowering the oxidative stress, inflammation, and hyper-insulinemia (Tarry-Adkins et al., 2016). Normally CoQ10 performs its regular function in electron transport chain (mitochondria), while acts as antioxidant in plasma and cell membranes where it prevents the lipid peroxidation (Ernster and Dallner, 1995). It restores the cellular functions of the antioxidant enzymes which are altered due to the oxidative stress induced by hydrogen peroxide and lowers the ROS production in response to it. It also enhances the defense capacity of the cellular antioxidants through both intrinsically scavenging the FR and activating the Nrf2; regulates the antioxidant expression in cells which protects from hydrogen peroxide induced oxidative toxicity (Fliedel et al., 2016).

1.2.3 Uric Acid

Uric acid is produced in humans as the end product of purine metabolism, the most copious antioxidant found in human plasma having robust scavenging activity for the carbon-centered and peroxyl radicals (Fabbbrini et al., 2014). It prevents the excess production of the Oxo-hem oxidants that leads to severe metabolic effects, formed during the reaction of peroxides and hemoglobin. Uric acid is also a potent scavenger of the FR mostly scavenges singlet oxygen and hydroxyl radicals thus prevents the lysis of erythrocytes by carrying out the peroxidation reactions (Carocho and Ferreira, 2013). Uric acid while acting as antioxidant also exerts its neuro-protective effect by decreasing the circulating (blood) concentrations of the malondialdehyde (MDA) and matrix metalloproteinase (MMP), the lipid peroxidation marker and blood-brain barrier marker respectively (Llull et al., 2016). Recent studies revealed that the level of uric acid is reduced in major depressive disorder (MDD), anxiety and autoimmune diseases, i.e. polymyositis and dermatomyositis (PM/DM) which leads to up-regulation of oxidative stress in the biological system (Black et al., 2016, Chen et al., 2017). A novel antioxidant mechanism is identified which uses uric acid to maintain the oxidants balance in body by p53-SLC2A9 pathway. SLC2A9 is a transporter of uric acid and associated with p53 gene which reduces the level of ROS in the biological system by transporting uric acid (Itahana et al., 2015). In the extracellular fluid, it remarkably scavenges the peroxynitrite (ONOO-) but for complete scavenging activity it requires ascorbic acid and thiols which specifies its crucial role in scavenging the peroxynitrite (Fig. 4) (Nimse and Pal 2015).
But irrespective of the protective properties of uric acid, its elevated level in the body commonly accompanied with higher risk for many diseases which includes cardiovascular disease and gout (Sautin et al., 2007). In a recent study it was concluded that uric acid also provides protection form the NSAID-induced enteropathy in the serum and intestinal lumen through its antioxidant action (Yasutake et al., 2017).

### 1.2.4 Glutathione

Glutathione (GSH) is a cysteine-containing tri-peptide, present in the cells cytosol in human body (Carocho and Ferreira, 2013). Cysteine is the sulfur containing amino acid and the thiol group (-SH) is responsible for its reducing action because it cycles between its oxidized form and reduced form (Birben et al., 2012). It is most important cellular antioxidant because of the presence of sulfhydryl group and high availability in biological system (Raffa et al., 2011). Three enzymes; glutathione reductase (GR), glutathione oxidase, and GSHPx are present in the GSH catalytic cycle hence, GSHPx and Glutathione oxidase converts GSH to GSSG by oxidation reactions while the regeneration of GSH from GSSG is carried out by glutathione reductase (GR) in an NADPH-dependent process (Fig. 5) (Raffa et al. 2011).
GSH in combination with these antioxidant enzymes provides a primary antioxidant defense mechanism against the harmful effects of ROS by maintaining cellular redox state (Baeza, Fdez-Tresguerres et al., 2010). GSH enhances ROS neutralization by its oxygen radical scavenging ability and helps in reducing the oxidative stress related damage to the cells (Nimse and Pal, 2015). GSH antioxidant system provides protection against the oxidant-induced cell death either autophagy or apoptosis, low levels of GSH is related to higher levels of oxidants and FR which consequently enhanced apoptosis.

1.2.5 Melatonin

Melatonin; "N-acetyl-5-methoxytryptamine", a indole containing neurohormone, synthesized in the pineal glands but large concentration are found in the mitochondrial portion of the cells, has many protective effects on various physiopathological functions (Rahman, 2007; Mathew et al., 2011). It is effective in reducing the oxidative stress in the body by reducing the pro-oxidant enzymes lipoxygenases. It directly scavenges ROS/RNS and increases the synthesis process of enzymatic antioxidants indirectly (Torres et al., 2015). Via its anti-radical mechanisms, it chelates the transition metals which carry out the Fenton/Haber-Weiss reactions, where it decreases the toxic hydroxyl radicals generation and toxicity of noxious prescription drug (Reiter et al., 2016). It has various distinctive anti-oxidative features as compared to other antioxidants, including the cascade pathway for scavenging numerous free radicals, because its secondary and
tertiary metabolites also neutralizes several harmful ROS/RNS derivatives (Pieri et al., 1994). One molecule of melatonin is capable of scavenging up to 10 molecules of ROS/RNS therefore, cascade reaction strengthens its capability as a potential antioxidant (Nohl et al., 2005). Melatonin is also useful in sickle cell anemia management either individually or in combination with N-acetyl cysteine (Silva et al., 2015) while N-acetyl cysteine is effective in schizophrenia and in pulmonary fibrosis (Brieger et al., 2012). It was also suggested that the derivate of melatonin having o-halogenated and di-halogenated aromatic side chain exhibits effectual antioxidant properties by significantly protecting the neuronal cells and scavenging ROS/RNS (Brewer 2011; Gurer-Orhan et al., 2016). Melatonin has extensive role in the immune and neuropsychiatric systems while it also regulates the bioenergetics (Zhao et al., 2019). Melatonin up-regulated the Nrf2 signaling pathway by lowering the oxidative damages due to FR and plays a potent role as neuroprophylactic against Aluminum-induced neurotoxic effect in rats (Sadek et al., 2019).

1.2.6 Vitamin C

Vitamin C is a water soluble substance generally known as ascorbic acid, acts as efficient antioxidant in the biological system and necessarily be obtained from exogenous sources in diet. It acts as a strong radical reducing agent in cell's cytoplasm (Limon-Pacheco and Gonsebatt, 2009) where it neutralizes ROS; it remains in reduced form by reacting with GSH in the cells. It works in combination with vitamin E to reduce ROS while regenerating vitamin E (reduced form) in the cell membranes and prevents from heart diseases (Pham et al. 2008; Sesso et al., 2008). It acts as oxygen scavenger by catalyzing the oxidation at low levels (<100 mg/kg) while it also maintains the balance between Fe$^{2+}$ and Fe$^{3+}$ by chelating the Fe$^{3+}$ ions (Brewer, 2011). It terminates the chain reaction of lipid peroxidation and in consequence to this termination it converts into ascorbate radical. When two ascorbate radicals reacts with each other one ascorbate and dehydroascorbate molecule is generated. Dehydroascorbate cannot contribute in the antioxidant system therefore it is transformed into ascorbate by addition of two electrons by oxidoreductase (Fig. 6) (Nimse and Pal, 2015).

![Fig. 6: Radical Scavenging Mechanism of Vitamin C (Nimse and Pal 2015)](image)

1.2.7 Vitamin E

Vitamin E is the lipid soluble substance, mostly abundant in plants and known as α-tocopherol. In biological system, it acts as potent antioxidant because it protects the cell membranes from lipid peroxidation reactions by quenching the lipids free radicals and their intermediates (Traber...
and Atkinson 2007; Kabel 2014). It terminates the peroxidation process by giving its phenolic hydrogen to peroxyl radicals, thus it acts as chain breaker in cell membranes (Carocho and Ferreira, 2013; Niki, 2014). The resultant tocopheroxyl radical is relatively stable under normal conditions and remains unreactive thus it cannot initiate lipid peroxidation chain reaction; vital criterion of a good antioxidant (Nimse and Pal, 2015). It can also inhibit oxidation of protein by reducing the formation of α-aminoacidipic and α-glutamic semi-aldehydes from oxidized myofibrillar proteins (Brewer, 2011). It was documented that α-tocopherol is utmost effective peroxyl radical scavenger in vitamin E family kinetically, and quantitatively, in human vitamin E is the major antioxidant in plasma and red blood cells (Stocker, 2016). Furthermore, high intake of vitamin E may be related to the decrease in the clinical progression of early Alzheimer’s disease symptoms and with a lower occurrence of Alzheimer’s disease (Sinyor et al., 2020).

\[ \alpha\text{-tocopherol–OH} + \text{LOO}^\cdot \rightarrow \alpha\text{-tocopherol–O}^\cdot + \text{LOOH} \]

### 1.2.8 Vitamin A

Vitamin A; retinol, is a carotenoid formed in the liver from the cleavage reaction of α-carotene by α-carotene-15,15α-dioxygenase enzyme. The antioxidant role of vitamin A was firstly identified by Monaghan and Schmitt, as it protects the lipids from rancidity. Its beneficial role is to protect the human low density lipoprotein from copper-stimulated oxidation (Nimse and Pal 2015). It combines with peroxyl radicals due to its radical scavenging activity and detoxify the toxic species before the radicals initiate the peroxidation chain reactions (Fig. 7) (Carocho and Ferreira, 2013).

![Fig. 7: Radical Scavenging Mechanism of Vitamin A](image)

### 1.2.9 Flavonoid

Flavonoids are group of compounds found in the plants and exhibits potential the antioxidant activities in the biological system when taken in through diet. These compounds share similar diphenylpropane \((C_6C_1C_6)\) skeleton and their antioxidant abilities are owing to the presence of phenolic groups, hence they works as superoxide radical scavengers, reducing agents, singlet oxygen quenchers, hydrogen donators and even as metal chelators (Carocho and Ferreira, 2013). The presence of multiple hydroxyl groups (-OH) enhances their antioxidant potential as compared to those compounds with has only one group and ortho- 3,4-dihydroxy moiety also increases their antioxidant activity (Brewer, 2011). Furthermore, they are also involves in activating the antioxidant enzymes,
lowering the oxidative stress due to ROS/RNS and increasing the levels of GSH, α-lipoic acid, CoQ 10 and uric acid in the body (Carocho and Ferreira, 2013). The hydroxyl groups at the C-5 and C-7 positions of flavonoids plays important role in xanthine oxidase (enzyme) inhibition because xanthine oxidase generates superoxide free radicals through catalysis of hypoxanthine and xanthine (Esatbeyoglu et al., 2017). Their indirect impact is to influence the gene expression of antioxidant enzymes by modulation of Nuclear Factor κB (NF κB) and Nuclear factor like 2 (Nrf2), redox sensitive transcription factors, that induces the activation of genes encoded for the antioxidant proteins (Dajas, 2016). Two new natural flavonoids were isolated from aerial parts of Ononis angustissima L., i.e. (3S)-7-hydroxy-4′-methoxy-isoflavanone 3′-β-d-glucopyranoside and kaempferol 3′-β-d-glucopyranoside-7-O-(2″′-acetyl)-β-d-galactopyranoside, which exhibits excellent antioxidant activity against ROS/RNS in the biological system (Mezrag et al., 2017).

1.2.10 Carotenoids

Carotenoids are lipid soluble colored compounds, present in plants and microorganisms. Conjugated double bonds are present in their structure which is responsible for their antioxidant activities due to delocalization of unpaired electrons. Antioxidant efficiency of the carotenoids for quenching the singlet oxygen and peroxo radicals is due to presence of conjugated double bonds in the molecule (Rahman, 2007). Carotenoids are important compounds because of their peroxyl radical scavenging activity by which they protect the cell membranes and lipoproteins from the oxidation reactions of free radicals. β-Carotenes are naturally occurring orange-colored carotenoid, exhibits potential antioxidant activity by quenching the singlet oxygen with higher efficiency (Brewer, 2011; Nimse and Pal, 2015; Imai et al., 2016). Lutein; most predominant carotenoids, is a eminent antioxidant and free radical scavenger with biological properties such as prevention of cardiovascular diseases (Ranard et al., 2017). Furthermore, in vivo antioxidant activities of β-carotene represented that it significantly reduces the oxidative stress (El-Baz et al., 2019).

1.2.11 S-allylcysteine (SAC)

SAC; organosulfur compound, is the most abundant water-soluble derivative of garlic and exhibits antioxidant property (Fig. 8) (Shi et al., 2015).

![Fig 8. Structure of S-allylcysteine (Shi, et al. 2015)](image)
SAC reduces oxidative stress and the accumulation of ROS, endoplasmic reticulum stress, mitochondrial dysfunction while enhances the functional activities of mitochondrial SODs, CAT, GSHPx, GST and the levels of GSH (Borek 2001; Colín-Gonzalez et al., 2015). SAC administration reduces the products of lipid peroxidation chain reaction and enhances the non-enzymatic antioxidants such as GSH, in the livers of diabetic rats (Naidu et al., 2016). Its exposure to the primary neurons provides protection against oxygen and glucose deprivation-induced oxidative insults and plays important role in activating the antioxidant Nrf2 signaling pathway in this way it protects the neurons from ischemic injury (Ashafaq et al., 2012; Shi et al., 2015). SAC was also involved in the up regulation of Heme oxygenase-1 (HO-1); defensive enzyme with anti-inflammatory property, through activation of the Nrf2 expression thus enhances its protective antioxidant effect (Baluchnejadmojarad et al., 2017). It was further evaluated that the derivatives of SAC also exhibited the neuroprotective effect which confirmed the antioxidant role of organosulfur compounds in biological system (Imai et al., 2016). SAC showed adequate therapeutic effect against diabetic nephropathy by reducing the oxidative stress and down regulation of inflammatory factors (Uddandrao et al., 2019).

CONCLUSION

Free radicals (FR) are the by-products of metabolic processes, associated with various physical and biochemical changes in human body. ROS/RNS induced oxidative stress leads to many deleterious effects including damage the proteins, lipids and DNA. If this stress exceeds the protection limit afforded by antioxidants then it subsequently increases the risk for various disorders such as cancer, inflammatory disorder, rheumatoid arthritis, cardiovascular disorder, diabetes etc. Therefore, balance between antioxidants and oxidants (FR) are crucial for the maintenance of biologic system. Naturally occurring antioxidants protects the body from the negative effects of FR and have beneficial impact on the human health. They may react directly with the ROS/RNS to eliminate their unpaired condition by donating electrons or they may indirectly decrease their formation either by lowering the expressions of enzymes that generate the free radicals or by enhancing the expressions of antioxidant enzymes which includes SOD, CAT, GSHPx, GSH and CoQ 10. Vitamin A, Vitamin E, Vitamin C, flavonoids and carotenoids are natural exogenous antioxidants which act as “chain breakers” for FR-induced oxidative reactions. The common feature of chain-breaking antioxidants is that they have one or more −OH groups which donates the H˙ to the oxidizing molecules. Flavonoids contains more than one aromatic ring and hydroxyl groups while ascorbic
acid and α-tocopherol each have only one aromatic ring thus have lesser hydroxyl groups in their structure and have positive impact in reducing effects of FR in the biological system. Level of exogenous antioxidants must be maintained through diet because the natural antioxidants play a significant role in defending the living beings from the harmful effects of the oxidants.

CONFLICT OF INTEREST STATEMENT

It is declared that authors have no conflict of interest.

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AUTHORS' CONTRIBUTIONS

MMS is involved in manuscript preparation along with MA; MARB in charge of ideas and supervision of this review article.

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