



# Free radical biology in cellular inflammation related to rheumatoid arthritis

A Bala<sup>1,2\*</sup>, PK Haldar<sup>1</sup>

## Abstract

### Introduction

Chronic inflammation is a prolonged pathological condition characterised by mononuclear cell infiltration, tissue destruction and fibrosis due to excess production of free radicals. Rheumatoid arthritis is systemic autoimmune disease characterised by synovial cell infiltration as well as destruction of tissues.

Free radicals are defined as molecules or any chemical species having an unpaired electron in the outer orbit and are capable of independent existence. In biological systems, the most common source of free radicals is oxygen which in term reacts with nitrogen and produces nitrogen intermediates. Under certain stress conditions, Oxygen becomes much more active and forms superoxide anion radicals and hydroxyl radical (OH<sup>-</sup>). The important nitrogen species are nitric oxide and peroxy-nitrite anion. Nicotinamide adenine dinucleotide phosphate oxidation by Nicotinamide adenine dinucleotide phosphate oxidase is considered as the major source of Superoxide anion radicals, where the antioxidant enzyme superoxide dismutase converts Superoxide anion radicals to less toxic hydrogen peroxide. Nitric oxides are formed from L-arginine by one of the three nitric oxide synthases

and react with O<sub>2</sub><sup>-</sup> and produce highly toxic Peroxynitrite. Collectively all reactive oxygen and nitrogen species are ultimately responsible for creating oxidative stress during inflammation by producing different cytotoxic cytokines through the phosphorylation of nuclear factor kappa-B (NFκB). The main aim of this review is to analyse the role of free radicals in cellular inflammation, related to rheumatoid arthritis.

### Conclusion

DNA damage caused by reactive radicals as well as cytokines may lead to abnormal functioning of the cells. Although cellular repair systems correct most of these damages but the free radicals induced DNA lesions can be an important aetiology of inflammatory autoimmune diseases like rheumatoid arthritis. Thus it should be recommended that management of oxidative stress by antioxidant therapies in combination with modulators of cytokines and other inflammatory pathways are the therapeutic weapon to fight against rheumatoid arthritis.

### Introduction

Inflammation is a protective defence mechanism employed by cells/tissues against endogenous and exogenous stimuli/antigens<sup>1</sup>. The relationship between chronic inflammation and rheumatoid arthritis (RA) has already been recognised<sup>2</sup>. RA is a systemic autoimmune disorder that primarily targets the synovium of diarthrodial joints resulting in an unchecked synovial inflammation<sup>3,4</sup> and accumulation of inflammatory cells like monocytes, macrophages and neutrophils in sinovium. In acute

inflammatory processes there is a marked accumulation of polymorphonuclear neutrophils however, chronic inflammation is a prolonged pathological condition characterised by mononuclear immune cell infiltration, tissue destruction and fibrosis due to excess production of reactive oxygen and nitrogen species (ROS and RNS) as well as toxic free radicals<sup>5</sup>. Chronic inflammation exerts its cellular side effects mainly through excessive production of free radicals and depletion of antioxidant defence in the body<sup>6</sup>. The main aim of this review is to analyse the role of free radicals in cellular inflammation, related to RA.

### What is free radical?

In the general structure of atoms and molecules, electrons are usually associated and exist in pairs, each pair moving within a defined region of space (an atomic or molecular orbital). One electron in each pair has a spin quantum number of + ½, the other - ½<sup>7</sup>. Free radicals are defined as molecules or any chemical species with an unpaired electron in the outer orbit and are capable of independent existence (hence the term 'free')<sup>8</sup>. This unpaired electron usually produces a highly reactive free radical.

In biological systems, the most common source of free radicals is oxygen which in term reacts with nitrogen and produces nitrogen intermediates and is termed as ROS and RNS<sup>9</sup>. Under certain stress conditions, Oxygen becomes much more active and forms ROS, such as superoxide anion radicals (O<sub>2</sub><sup>-</sup>), hydroxyl radical (OH<sup>-</sup>), peroxy radical (ROO<sup>-</sup>)

\* Corresponding author  
Email: asisbala\_ju@yahoo.co.in

<sup>1</sup>Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, West Bengal, India

<sup>2</sup>Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F Nillgunj Road, Panihati, Sodepur, Kolkata 700114, West Bengal, India

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

**FOR CITATION PURPOSES:** Bala A, Haldar PK. Free radical biology in cellular inflammation related to rheumatoid arthritis. OA Arthritis 2013 Aug 01;1(2):15.

and non free-radical species, such as  $H_2O_2$  and singlet oxygen ( $^1O_2$ )<sup>10</sup>. The most important RNS are nitric oxide (NO), nitrogen dioxide ( $NO_2$ ) and peroxy nitrite anion<sup>11</sup>. Thus ROS or RNS are produced in human cells under physiologic and pathologic conditions and both include radical and non-radical species<sup>12</sup>.

### Production of free radicals

Free radicals and other reactive species are constantly generated in the human body. Oxygen is required for the generation of all ROS and RNS. Initially Superoxide ( $O_2^-$ ) is generated from  $O_2$  by multiple pathways (Table 1).

Out of which nicotinamide adenine dinucleotide phosphate (NADPH) oxidation by NADPH oxidase is considered as the major source of  $O_2^-$  in the living cells. The antioxidant enzyme superoxide dismutase (SOD) converts  $O_2^-$  to less toxic hydrogen peroxide ( $H_2O_2$ ). Hydrogen peroxide is also produced through two-electron reduction of  $O_2$  by cytochrome P-450, D-amino acid oxidase, acetyl coenzyme A oxidase or uric acid oxidase<sup>12,14</sup>. In the presence of water and oxygen, ionising radiation results in the production of  $O_2^-$  and  $H_2O_2$ <sup>17</sup>. Subsequently HOCl is generated from  $H_2O_2$  and  $Cl^-$  by a heme enzyme myeloperoxidase (MPO) particularly in immunologically activated macrophages cells. Surprisingly during respiratory burst, neutrophils,

monocytes and macrophages generate superoxide and its subsequent dismutation generates  $H_2O_2$  that produces the toxic hydroxyl radical<sup>18</sup> via a  $Fe^{++}$  mediated reaction which is known as Fenton reaction.

Nitric oxide (NO) is formed from L-arginine by one of the three Nitric oxide synthase (NOS) isoforms: nNOS (originally identified as constitutive in neuronal tissue; also known as NOS-I or NOS-1), iNOS (originally identified as being inducible by cytokines in activated macrophages and liver; also known as NOS-II or NOS-2) and eNOS (originally identified as constitutive in vascular endothelial cells; also known as NOS-III or NOS-3)<sup>16</sup>. All NOS isoforms require oxygen, tetrahydrobiopterin, NADPH, calmodulin, flavin adenine dinucleotide (oxidised; FAD), flavin mononucleotide (FMN) and heme for catalytic activity, whereas  $Ca^{2+}$  is essential for nNOS and eNOS activity<sup>18</sup>. Additionally NO can react with  $O_2^-$  or  $H_2O_2$  to form peroxynitrite ( $ONOO^-$ ), whose oxidant potential is greater than that of  $O_2^-$  or  $H_2O_2$  alone probably due to inability to escape from the cells as its molecular weight is high<sup>14,19</sup>.

### Effect of Free radicals

Generally some important physiological functions are regulated by redox-responsive signalling pathways through the production of NO by NOS and oxygen radical ( $O_2^-$ ) production

by phagocytic NAD(P)H oxidase<sup>20</sup>. Additionally gene transcription and regulation of soluble guanylate cyclase activity in cells is also regulated by ROS and RNS. Furthermore, NO is one of the most widespread signalling molecules and participates in virtually every cellular and organ function in the body mainly as endothelial dependent relaxing factor (EDRF)<sup>21</sup>. Physiologic levels of NO produced by endothelial cells are essential for regulating the relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, vascular tone and haemodynamics. NO produced by neurons serves as a neurotransmitter and NO generated by activated macrophages is an important mediator of the immune response<sup>22</sup>.

However, due to having an extra electron in the outer orbital, free radicals and other reactive species play a critical 'super oxidant' role to cause the oxidation of biomolecules mainly protein, amino acids, lipid and DNA, which leads to cell injury and death<sup>14,19</sup>. The cell membrane is one of the most susceptible sites for ROS/RNS induced damage. Free radicals can react with cell membrane fatty acids and form lipid peroxides. Lipid peroxides accumulation can lead to production of carcinogenesis agents like malondialdehyde<sup>23</sup>. These changes are particularly significant in long-lived cells such as neurons<sup>24</sup>.

**Table 1 Multiple pathways of  $O_2^-$  generation**

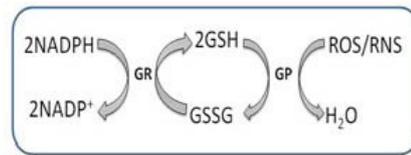
1) NADPH oxidation by NADPH oxidase in reaction with oxygen <sup>2</sup> .
2) Sometimes made by 'accidents of chemistry'; leakage of electrons directly on to $O_2$ from the intermediate electron carriers of the mitochondrial electron transport chain generates a steady stream of $O_2^-$ <sup>7</sup> .
3) Exposure of living organisms to ionising radiation <sup>13</sup> .
4) Oxidation of xanthine or hypoxanthine by xanthine oxidase <sup>8</sup> .
5) Oxidation of reducing equivalents (e.g. NADH, NADPH and $FADH_2$ ) via the mitochondrial electron transport system <sup>12</sup> .
6) Autoxidation of monamines (e.g. dopamine, epinephrine and norepinephrine), flavins and hemoglobin in the presence of trace amounts of transition metals <sup>14</sup> .
7) One-electron reduction of $O_2$ by cytochrome P-450 <sup>15</sup> .
8) One-electron reduction of $O_2$ by nNOS or eNOS when arginine or tetrahydrobiopterin is deficient <sup>16</sup> .

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

The proteins are other main targets for free radicals attack. Overproduced radicals can react with protein amino acids to oxidise and cross-link them. Radical-protein reactions can impair the function of important cellular and extracellular proteins like enzymes and connective tissue proteins permanently<sup>25</sup>. DNA is also highly susceptible to free radical attacks. An oxygen radical interaction with DNA can break its strands or delete a base. This DNA damage can be a lethal event for an organism. The rate of DNA damage inflicted by free radicals is considerably high; it is estimated that on average of more than 10,000 oxidative hits occur each day in the DNA of human cells<sup>26</sup>.

### Antioxidant defences in our body

Antioxidant enzymes are considered as the first line of cellular defence against oxidative damage. Among the antioxidant enzymes, catalase (CAT), GSH peroxidases (GP), superoxide dismutase (SOD) and antioxidant nutrients are the most important antioxidant defence components in cells exposed to oxygen, whereas reduced glutathione (GSH) is the most important non enzymatic antioxidant defence<sup>10</sup>. The tripeptide glutathione in its reduced form (GSH; c-L-glutamyl-L-cysteinyl-glycine) by virtue of its free radical scavenging activity and reduction of peroxides is considered as the principal cellular antioxidant<sup>27</sup>. Glutathione, the most abundant thiol-containing substance of low molecular weight in cells, is synthesised from glutamate, cysteine and glycine. N-acetylcysteine is a stable, effective precursor of cysteine for intracellular GSH synthesis<sup>28</sup>. As a major component of the cellular antioxidant system, GSH (reduced) react with all ROS and RNS and stabilise them by donating H<sup>+</sup> and itself converted to GSSG (oxidised glutathione) (Figure 1). GSSG again get back to GSH by the action of glutathione reductase (GR) with the coenzyme system NADH<sup>29</sup>.



**Figure 1:** Role of GSH as an antioxidant.

### Pharmacology of inflammation and arthritis

Inflammation is the early response of the body's immune system to eliminate pathogens or other stimuli in order to restore the cells to normal state or replace destroyed tissue with scar<sup>30</sup>. The interaction of the cellular immune system with endogenous and/or exogenous antigens results in increased generation of ROS and RNS, leading to the activation of signalling cascades of synthesising proinflammatory cytokines and chemokines<sup>30,31</sup>. In the innate immune system, macrophages play a pivotal role in eliminating the pathogen through the generation of ROS/RNS including superoxide, nitric oxide, hydrogen peroxide, hydroxyl radical, ONOO<sup>-</sup> and hydrochlorous acid (HOCl)<sup>31</sup>.

During inflammation Cyclooxygenase (COX) is the main enzyme that is unregulated in the macrophages<sup>32</sup>. COX is an enzyme that is responsible for the formation of important biological mediators called prostanoids (including prostaglandins, prostacyclin and thromboxane). COX converts arachidonic acid to prostaglandins which can result in pain, swelling and stiffness<sup>33</sup>. COX exists in two isoforms, COX-1 and COX-2; COX-1 is constitutive while COX-2 is inducible and is produced in abundance in activated macrophages and other cells at the site of inflammation. COX-2 derived bioactive lipids, including prostaglandin E<sub>2</sub>, are potent inflammatory mediators. PGE<sub>2</sub> increases vascular permeability along with other vasoactive components such as histamine, bradykinin or nitric oxide thereby causing oedema, pain and hyperalgesia at the local inflammatory

sites<sup>34</sup>. Synthesis of prostaglandins E<sub>2</sub> (PGE<sub>2</sub>), an oxygenated product of arachidonic acid, is dependent on ROS and NO mediated activation of COX, thus several anti-inflammatory compounds also display antioxidant properties and vice versa<sup>35</sup>.

Studies indicate that Th1 cytokines can promote the development of autoimmune disorders like rheumatoid arthritis (RA), whereas the Th2 pattern may attenuate these diseases<sup>36</sup>. The Th1 and Th2 cytokine balance have attracted great interest as it is hypothesised that the degree of polarisation and heterogeneity of T cell lymphocytes may be important in the initiation and perpetuation of inflammation in arthritis<sup>34</sup>. Recent studies have revealed key roles for inflammatory cytokines, such as tumour necrosis factor alpha (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$  and IL-6, in pathogenesis of arthritis. Levels of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 are elevated in the synovium of patients with arthritis<sup>37</sup>. It has been suggested that these inflammatory cytokines are produced through continuous activation of T cells and interaction of activated T cells with monocytes/macrophages in arthritis<sup>38</sup>.

### Free radicals in inflammation and rheumatoid arthritis

Many studies have demonstrated the direct relationship between chronic inflammation and rheumatoid arthritis. Cytokines are soluble mediators of intracellular communications which are upregulated during inflammation. They contribute to a chemical signalling language that regulates development, tissue repair, haemopoiesis, inflammation and the specific and non-specific immune responses<sup>28</sup>. Rheumatoid Arthritis (RA) is a systemic autoimmune disorder that primarily targets the synovium of diarthrodial joints resulting in an unchecked synovial inflammation that leads to erosions of periarticular surfaces and juxta-articular osteopenia<sup>2</sup>. Though the

precise aetiology of RA remains unknown, studies have implicated a role for oxidative stress and redox signalling in its pathogenesis<sup>39</sup>. Oxidative stress is the ultimate potential biomarker for determining disease activity in patients with RA<sup>39</sup>. Higher amounts of ROS and RNS have been reported in the synovial joints as well as peripheral blood inflammatory cells of the RA patients<sup>38</sup>. The disease is consistently associated with an increase in various proinflammatory factors that include cytokines (IL-1  $\beta$ , IL-6, tumour necrosis factor alpha TNF- $\alpha$ ), prostaglandins, reactive oxygen species (ROS) and nitric oxide (NO) at sites of inflammation, coupled with very low concentrations of superoxide dismutase (SOD) in the synovial fluid<sup>39</sup>.

ROS/RNS and inflammatory cytokines, like TNF- $\alpha$  activate a transcription factor called nuclear factor kappa-B (NF $\kappa$ B) by phosphorylation and subsequent proteasomal degradation. After that, NF $\kappa$ B migrates to the nucleus and activate specific gene transcription<sup>33</sup>. NF $\kappa$ B induces the expression of genes involved in cell proliferation, apoptosis and carcinogenesis<sup>39</sup>. NF $\kappa$ B can also induce production of proinflammatory cytokines, which will enhance the inflammatory responses. In normal state NF $\kappa$ B is inhibited by its inhibitory protein (I $\kappa$ B) which can down regulate the inflammatory response<sup>40</sup>.

Peroxynitrite (ONOO<sup>-</sup>) can diffuse within cells and cause damage during chronic inflammation<sup>39,41</sup>. The less reactive molecules such as nitric oxide (NO) can be released from innate immune cells especially macrophages and act on neighbouring cells, leading to somatic mutations and cancer<sup>40</sup>. Nitric oxide can react with superoxide and form ONOO<sup>-</sup>. This reactive intermediate can induce oxidative DNA damage including breaking of single and double strands, releasing of free nucleobases, chemical changes of

nucleobases and modification of sugar moieties. Ultimately DNA damage may lead to abnormal functioning of the cell<sup>33</sup>. Although cellular repair system corrects most of these damages, the free radicals induced DNA lesions that accumulated with age, can be an important aetiology of inflammatory autoimmune diseases like rheumatoid arthritis<sup>41</sup>.

### Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects in these referenced studies gave informed consent to participate in these studies.

During the last decade, there has been an escalation of interest in the role of antioxidants in health care and disease. Antioxidants act as free radical scavengers and are thus found to play a significant protective role against oxidative stress in a variety of diseases such as liver cirrhosis, inflammation, atherosclerosis, diabetes, cancer, neurodegenerative diseases and also in rheumatoid arthritis<sup>40</sup>. Many important molecules including proteins, lipids and nucleic acid chains are very susceptible to oxidising reactions by free radicals<sup>42</sup>; thus, it is not expected to see many deteriorating events following oxidative stress leading to decreased longevity of the cells as well as destroying the normal physiological function of cells<sup>42</sup>. Pro-inflammatory cytokines are key elements in abnormal transformation of the cells and auto immune activities leading to RA thus chronic inflammation should be considered as a high risk factor for RA. The antioxidant defines

system within the body can easily handle free radicals that are internally produced<sup>43</sup>. Antioxidants are manufactured within the body and can also be extracted from the food that are consumed by humans such as fruits, vegetables, seeds, nuts, meats and oil. The principle micronutrient antioxidants are vitamin E, beta-carotene and vitamin C<sup>43,44</sup> where the body's nonenzymatic potent antioxidant is GSH<sup>45</sup>.

### Conclusion

Based on these analogies, it should be recommended that management of oxidative stress by antioxidant therapies in combination with modulators of cytokines and other inflammatory pathways are the therapeutic weapon to fight against rheumatoid arthritis.

### Abbreviations list:

CAT, Catalase; COX, Cyclooxygenase GP, GSH peroxidises; GSH, Reduced glutathione; GSSG, Oxidised glutathione; IL, Interleukin; NF $\kappa$ B, Nuclear factor kappa-B; O<sub>2</sub><sup>-</sup>, Superoxide anion radicals; OH<sup>-</sup>, Hydroxyl radical; ONOO<sup>-</sup>, Peroxynitrite; PGE<sub>2</sub>, Prostaglandins E<sub>2</sub>; RA, Rheumatoid arthritis; RNS, Reactive nitrogen species; ROO<sup>-</sup>, Peroxyl radical; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TNF- $\alpha$ , Necrosis factor alpha.

### Acknowledgement

I would like to thank Dr. Abhijit Sengupta, Director cum Principal, Guru Nanak Institute of Pharmaceutical Science and Technology for his valuable inspiration to write this review article.

### Reference

- Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol*. 2008 Apr;66(1): 1-9.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

2. Kundu S, Bala A, Ghosh P, Mitra A, Sarkar A, Bauri AK, et al. Attenuation of oxidative stress by allylpyrocatechol in synovial cellular infiltrate of patients with Rheumatoid Arthritis. *Free Radic Res.* 2011 May;45(5):518–26.
3. Kundu S, Ghosh P, Datta S, Ghosh A, Chattopadhyay S, Chatterjee M. Oxidative stress as a potential biomarker for determining disease activity in patients with rheumatoid arthritis. *Free Radic Res.* 2012 Dec;46(12):1482–9.
4. Feely MG, Erickson A, O'Dell JR. Therapeutic options for rheumatoid arthritis. *Expert Opin Pharmacother.* 2009 Sep;10(13):2095–06.
5. Magari K, Miyata S, Ohkubo Y, Mutoh S. Inflammatory cytokine levels in paw tissues during development of rat collagen-induced arthritis: effect of FK506, an inhibitor of T cell activation. *Inflamm Res.* 2004 Sep;53:469–74.
6. Hold GL, El-Omar ME. Genetic aspects of inflammation and cancer. *Biochem J.* 2008 Mar;410(2):225–35.
7. Gutteridge JMC, Halliwell B. The deoxyribose assay: an assay both for free hydroxyl radical and for site specific hydroxyl radical production. *Biochem J.* 1988 Aug;253(3):932–3.
8. Gilbert DL. Fifty years of radical ideas. *Ann NY Acad Sci.* 2000;899:1–14.
9. Salvemini D, Cuzzocrea S. Oxidative stress in septic shock and disseminated intravascular coagulation. *Free Radical Bio Med.* 2002 Nov;33(9):1173–85.
10. Halliwell B. Reactive oxygen species and the central nervous system. *J Neurochem.* 1992 Nov;59(5):1609–23.
11. Cotellet N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antioxidant properties of hydroxy-flavones. *Free Rad Biol Med.* 1996;20(1):35–43.
12. Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. *Br J Nutr.* 2001 May;85(Suppl 2):S67–74.
13. Reilly PM, Schiller HJ, Bulkey GB. Pharmacologic approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. *Am J Surg.* 1991;161:488–5.
14. Freidovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? *Ann NY Acad Sci.* 1999;893:13–8.
15. Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med.* 1999 Nov;27(9–10):916–21.
16. Wu G, Morris SM. Arginine metabolism: nitric oxide and beyond. *Biochem J.* 1998 Nov;336(Pt 1):1–17.
17. Fang YZ. Effect of ionising radiation on superoxide dismutase in vitro and in vivo. In: Fang YZ, editors. (English edition). *Advances in free radical biology and medicine.* Beijing: Atomic Energy Press; 1991;1:p1.
18. Sarna LK, Wu N, Hwang SY, Siow YL, OK. Berberine inhibits NADPH oxidase mediated superoxide anion production in macrophages. *Can J Physiol Pharmacol.* 2010 Mar;88(3):369–78.
19. McCord JM. The evolution of free radicals and oxidative stress. *Am J Med.* 2000 Jun;108(8):652–9.
20. Dröge W. Free radicals in the physiological control of cell function. *Physiol Rev.* 2002 Jan;82(1):47–95.
21. Halliwell B. Reactive oxygen species in living systems: a thorough explanation of free radicals source: biochemistry and role in human disease. *Am J Med.* 1991 Sep;91(3C):14S–22S.
22. Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol.* 1999 Dec;34(6):879–86.
23. Lippman RD. Lipid peroxidation and metabolism in aging. In: Rothstein M, editors. *Review of biological research in aging.* New York: Alan R. Liss. 1983;1:p315–42.
24. Ran O, Gu M, Van Remmen H. Glutathione peroxidase 4 protects cortical neurons from oxidative injury and amyloid toxicity. *J Neurosci Res.* 2006 Jul;84(1):202–8.
25. Stadtman ER. The status of oxidatively modified proteins as a marker of aging. In: Esser K, Martin GM, editors. *Molecular aspects of aging.* Chichester: Wiley; 1995.p129–44.
26. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. *Proc Natl Acad Sci U S A.* 1993 Sep;90(17):7915–22.
27. Price M, Reiners JJ, Santiago AM, Kessel D. Monitoring singlet oxygen and hydroxyl radical formation with fluorescent probes during photodynamic therapy. *Photochem Photobiol.* 2009 Sep–Oct;85(5):1177–81.
28. Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med.* 1999 Nov;27(9–10):916–21.
29. Johnson FB, Sinclair DA, Guarente L. Molecular biology of aging. *Cell.* 1999;96(2):291–302.
30. Emmendoerffer A, Hecht M, Boeker T, Mueller M, Heinrich U. Role of inflammation in chemical-induced lung cancer. *Toxicol Lett.* 2000 Mar;112–113:185–91.
31. Ryan KA, Smith MF Jr, Sanders MK, Ernst PB. Reactive oxygen and nitrogen species differentially regulate Toll-like receptor 4-mediated activation of NF-kappa B and interleukin-8 expression. *Infect Immun.* 2004 Apr;72(4):2123–30.
32. Flalkow L, Wang Y, Downey GP. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. *Free Radic Biol Med.* 2007 Jan;42(2):153–64.
33. Sarkar D, Saha P, Gamre S, Bhattacharjee S, Hariharan C, Ganguly S, et al. Anti-inflammatory effect of allylpyrocatechol in LPS-induced macrophages is mediated by suppression of iNOS and COX-2 via the NF-kappaB pathway. *Inter Immunopharmacol.* 2008 Sep;8(9):1264–71.
34. Magari K, Miyata S, Ohkubo Y, Mutoh S. Inflammatory cytokine levels in paw tissues during development of rat collagen-induced arthritis: effect of FK506, an inhibitor of T cell activation. *Inflammation Res.* 2004 Sep;53(9):469–74.
35. Mollace V, Muscoli C, Masini E, Cuzzocrea S, Salvemini D. Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors. *Pharmacol Rev.* 2005 Jun;57(2):217–52.
36. Pandey A, Bani S, Dutt B, Suri KA. Modulation of Th1/Th2 cytokines and inflammatory mediators by hydroxychavicol in adjuvant induced arthritis tissues. *Cytokine.* 2010;49:114–21.
37. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol.* 1996;14:397–440.
38. Feldmann M, Maini RN. Anti-TNF-alpha therapy of rheumatoid arthritis: what have we learned? *Annu Rev Immunol.* 2001;19:163–96.
39. Mirshafiey A, Mohsenzadegan M. The role of reactive oxygen species in immunopathogenesis of rheumatoid arthritis. *Iran J Allergy Asthma Immunol.* 2008 Dec;7(4):195–202.
40. Hitchon CA, El-Gabalawy HS. Oxidation in rheumatoid arthritis. *Arthritis Res Ther.* 2004;6(6):265–78.
41. Wickens AP. Ageing and the free radical theory. *Respir Physiol.* 2001 Nov;128(3):379–91.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

**FOR CITATION PURPOSES:** Bala A, Haldar PK. Free radical biology in cellular inflammation related to rheumatoid arthritis. *OA Arthritis* 2013 Aug 01;1(2):15.

42. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Pat Inflamm Allergy Drug Discov.* 2009 Jan;3(1):73–80.
43. Bonnefont-Rousselot D. Glucose and reactive oxygen species. *Curr Opin Clin Nutr Metab Care.* 2002 Sep;5(5):561–8.
44. Funda SP, Kiyomet T. Free radicals: our enemies or friends? *Adv Mol Biol.* 2007;1:63–9.
45. Bala A, Chetia P, Dolai N, Khandelwal B, Haldar PK. Cat's whiskers flavonoid attenuated oxidative DNA damage in macrophages cells: Its Importance in patients with Rheumatoid Arthritis. *Inflammopharmacology.* 2014 Feb;22:55–61.