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Review Article

Reactive oxygen species : A key hallmark of cardiovascullar disease

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ABSTRACT

Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. They are produced either from normal cell metabolisms *in situ* or from external sources (pollution, cigarette smoke, radiation, medication). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases.

Cardiovascular diseases (CVDs) have been the prime cause of mortality worldwide for decades. However, the underlying mechanism of their pathogenesis is not fully clear yet. It has been already established that reactive oxygen species (ROS) play a vital role in the progression of CVDs. ROS are chemically unstable reactive free radicals containing oxygen, normally produced by xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidase, lipoxygenases or mitochondria or due to the uncoupling of nitric oxide synthase in vascular cells. When the equilibrium between production of free radicals and antioxidant capacity of human physiology gets altered due to several pathophysiological conditions, oxidative stress is induced, which in turn leads to tissue injury. The information generated by this review aims to provide updated insights into the understanding of the mechanisms behind cardiovascular complications mediated by ROS.

Figures : 02 References : 54 Table : 00

KEY WORDS : Antioxidants, Cardiomyopathy, Cardiovascular diseases, Free radicals, Heart failure.

Introduction

Our atmosphere: 'air vital' and 'gas azote'

Chemist[23], was the first to recognize that Earth's atmosphere was composed of substances ('air vital') that supported life. Oxygen, as the key life-supporting element, was independently discovered[37,39]. Within a few years of these seminal findings, it was discovered that oxygen had toxic side effects that did not support life ('gas azote'). This revelation was also made by a simple (perhaps ingenious at the time) experiment in which guinea pigs exposed to oxygen in a container showed congestion of the right heart as well as lungs and died before the oxygen was fully utilized[24]. Thus, the discoverers of oxygen, more than two centuries ago, already knew about the good and bad facets of oxygen. About two centuries later, the discovery of an important antioxidant enzyme, superoxide dismutase, renewed interest in oxygen-radical biology[30].

Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. These species play a dual role as both toxic and beneficial compounds. The delicate balance between their two antagonistic effects is clearly an important aspect of life. At low or moderate levels, ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures[2,8,10,13,14,32,35,48,52]. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ* or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as "free radical scavengers" by preventing and repairing damages caused by ROS and RNS and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases[6,9,34,46-48].

The theory of oxygen-free radicals has been known about fifty years ago[48]. However, only within the last two decades, has there been an explosive discovery of their roles in the development of diseases and also of the health protective effects of antioxidants.

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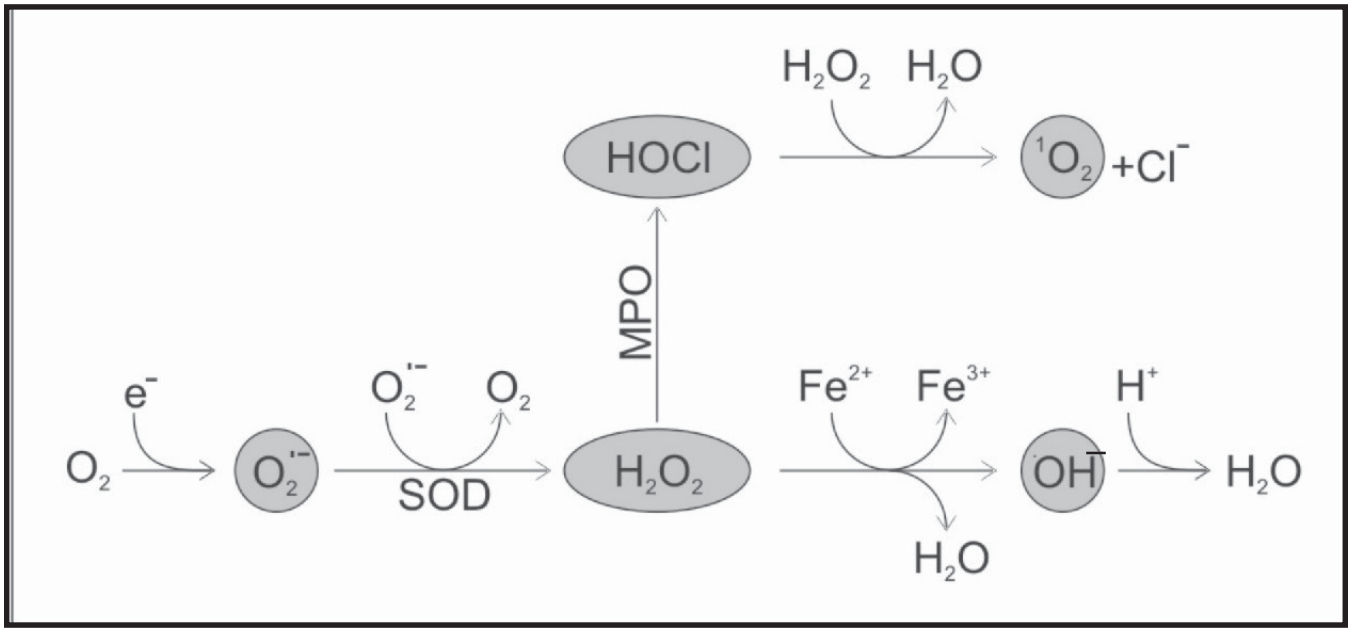


Fig. 1 : Production of ROS. The figure shows the pathway of ROS production in the human body with various enzymes involved. SOD : superoxide dismutase; MPO: myeloperoxidase.

Chemical Characteristics of Reactive Oxygen Species (ROS)

Researchers have been continuously studying the potential role of oxidative damage in cardiovascular diseases (CVDs) for a few decades. In a simple term, the common risk factors for CVDs like diabetes mellitus, smoking, aging, hypercholesterolemia and nitrate intolerance can further increase the possibility of the generation of ROS. Furthermore, these risk factors can trigger several pathways such as apoptosis of endothelial cells (EC), expression of adhesion molecules, activation of metalloproteinases, induction of proliferation and migration of smooth muscle cells, lipid peroxidation and change in vasomotor functions, collectively leading to CVDs[33,49]. ROS are chemically reactive molecules containing oxygen. Several ROS with unpaired electrons, for instance, superoxide anion, hydroxyl radical, and lipid radicals, are considered as free radicals. ROS, such as hydrogen peroxide (H_2O_2), peroxynitrite (ONOO), and hypochlorous acid (HOCl), are not free radicals but possess an oxidizing effect resulting in oxidant stress. A chain reaction leads to the production of many reactive oxygen species from one ROS (Figure 1). For example, the reactions of radicals and fatty acids (polyunsaturated fatty acids, PUFAs) within the cytoplasmic membrane result in a fatty acid peroxyl radical which can attack the adjacent side chain of the fatty acid and commence production of other lipid radicals. Lipid radicals generated in this chain reaction get collected in the plasma membrane and may have innumerable effect on cell function, including alteration in cell membrane permeability and dysfunction of membrane-bound receptors[3,49].

Potential Sources of ROS for CVDs

In a physiological system, the imbalance between antioxidant defense mechanism and ROS production leads to oxidative stress and subsequent pathological conditions[19]. Most prominent ROS causing toxic insult to the human body are H_2O_2 and ONOO[54]. In the blood vessel wall, each layer can produce ROS in pathological conditions[38]. Workers[50] reported that, within mitochondria, oxygen is usually utilized for energy production (in the form of ATP) and oxidative phosphorylation. During the mitochondrial electron transport (MET), harmful ROS are formed but they are balanced by antioxidant defense. However, in case of ischemia or hypoxia, MET is imbalanced, leading to ATP depletion, acidosis, mitochondrial depolarization, collection of noxious metabolites, intracellular Ca^{2+} overload, and cell death[50]. For example, approximately 1–3% of molecular oxygen is converted to unstable/ reactive in mitochondrial complexes I and III through a pathway involving oxidative phosphorylation[31]. In general, cardiac myocytes consume a high level of oxygen due to considerable higher number of mitochondria than other cells[53].

For this reason, cardiac myocytes also release ROS and cause oxidative stress to other cells[15]. But ROS do not have only a negative side, since production of ROS at physiological levels promote cellular activities, control the hormone level, maintain chemical balance, strengthen synaptic plasticity and induce enzymes. Moreover, ROS also helps to fight against invading pathogens and induce

an immune response against the pathogenic influence[54]. To a certain extent, ROS are neutralized by intracellular antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase and consumption of other nonenzyme antioxidants like *â*-carotene, ascorbic acid, and tocopherols as a supplement[50]. In spite of being necessary to carry out cell signaling pathways, overproduction of ROS lead to injury of the cell membrane integrity causing altered permeability, change in proteins expression and DNA damage[43]. For the majority of CVDs, the enzymatic sources of ROS include NAD(P)H oxidase, lipooxygenase, cyclooxygenase (COX), xanthine oxidase (XO), uncoupled nitric oxide synthases (NOS), cytochrome P450 and mitochondrial respiration[7,35,40] (Figure 2). The process of increased O_2^- generation, facilitated by XO enzyme, can be antagonized by a therapeutic approach with XO inhibitor, like allopurinol, to ameliorate cardiac conditions[4]. NADPH oxidase (Nox), commonly found on the cellular membrane, is stimulated during phagocytosis leading to increased ROS release[15]. In particular, the over expression of Nox_2 and Nox_4 is linked to the remarkable oxidative stress observed during CVDs. A study[22] showed that Nox_4 knockout mice showed a low level of cardiac revealing that Nox_4 is a potential source of superoxide in cardiac myocytes. Nox_4 over expression worsened the cardiac function and induced apoptosis and fibrosis in a mouse with response to pressure overload. Thus, Nox_4 is a key contributor of oxidative stress in the mitochondrial redox systems leading to cardiac impairment during pressure overload. Therefore, the physiological role of Nox, translocating electrons throughout the membrane, can be deregulated in CVDs leading to cardiac dysfunction[22]. However, some pathways associated with ROS mediated CVDs are yet to be clarified. However, researchers are trying to reveal good, bad and ugly roles of ROS in the physiological system. In contrast to the good face of ROS on signaling and immune response at high concentrations, ROS can exhibit the deleterious effect on redox homeostasis leading to intracellular components damage as seen in neurodegenerative diseases, CVDs, and pulmonary disorders[54].

Reactive Oxygen Species and Atherosclerosis

Excess production of ROS plays an important role in inflammation, disturbed blood flow/abnormal shear stress and arterial wall remodeling. ROS cause remodeling through proliferation of smooth muscle cell and increased inflammation[18]. Repeated continuous exposure to nonstreamline shear stress of arterial regions generates O_2 induced by endothelial Nox resulting in adhesion of monocytes[20]. The upregulation of adhesion molecules including P-selectin, VCAM-1, and E-selectin causes further inflammation by adhesion of white blood cells. Development of inflammatory response increases ROS production by phagocytosis, which is important in the early stage of atherosclerosis[25, 36]. The Nox family of superoxide producing proteins is an important source of ROS in signal transduction. Nox are found to be expressed in phagocytic cells, EC, smooth muscle cells, and fibroblasts. Experiments conducted on arteries from human volunteers with coronary artery disease and animal experimental model with hypertension, diabetes, or atherosclerosis demonstrated that Nox_1, Nox_2, and Nox_5 stimulate endothelial dysfunction, inflammation and programmed cell death; however, isoform Nox_4 protects the vascular system by increasing bioavailability of nitric oxide and stoppage of cell death pathways[44]. Some research presents the controversial role of Nox_4 displaying either protective or a deleterious role of Nox_4.

Nox_4 are found abundantly in kidney, vascular cells, and osteoclasts[22]. Angiotensin II type 1 receptor activation and hypertension are linked to increased expression of Nox_1 and Nox_4 that could lead to vascular damage during chronic hypertension[1]. MCP-1 is essential for the formation of endothelial cell tumors (hemangioendotheliomas) which is redox sensitive. It was found that only the Nox_4 isoform was present in endothelial cell tumors cells whereas knockdown of *Nox_4* gene remarkably decreased the expression of MCP-1 as well as hemangioendothelioma formation. This was due to the fact that, in hemangioendothelioma cells, Nox_4 delivers H_2O_2 to the nuclear compartment causing oxidative alteration of DNA[11]. Inflammation mediates all stages of atherosclerosis and ROS sources might include infiltrated monocytes/macrophages, dysfunctional EC, and smooth muscle cells that migrated from tunica media to tunica intima layers of the wall of an artery. ROS oxidized-LDL is available in the arterial wall and macrophages scavenge it resulting in the formation of foam cells. This is one of the important steps in the progression and development of atherosclerosis[16]. Also, the calcium-dependent zinc containing endopeptidase, matrix metalloproteinase, secreted from EC, foam cells, and vascular smooth cells, is activated during oxidative stress in part due to inflammation and nonlaminar shear stress, resulting in the ruptures of thrombosis[17].

Role of Antioxidants and oxidative stress in CVDs

The role of antioxidants and oxidative stress in cardiovascular diseases has been described[41]. It has been reported that increased intake of antioxidants such as vitamins C and E, protects cardiovascular diseases. However, irrational or excessive use of antioxidants may produce risk of potential toxicity. The highly reactive oxygen derived free radicals (ROS) of endogenous or environmental origin play a cognitive role in the genesis

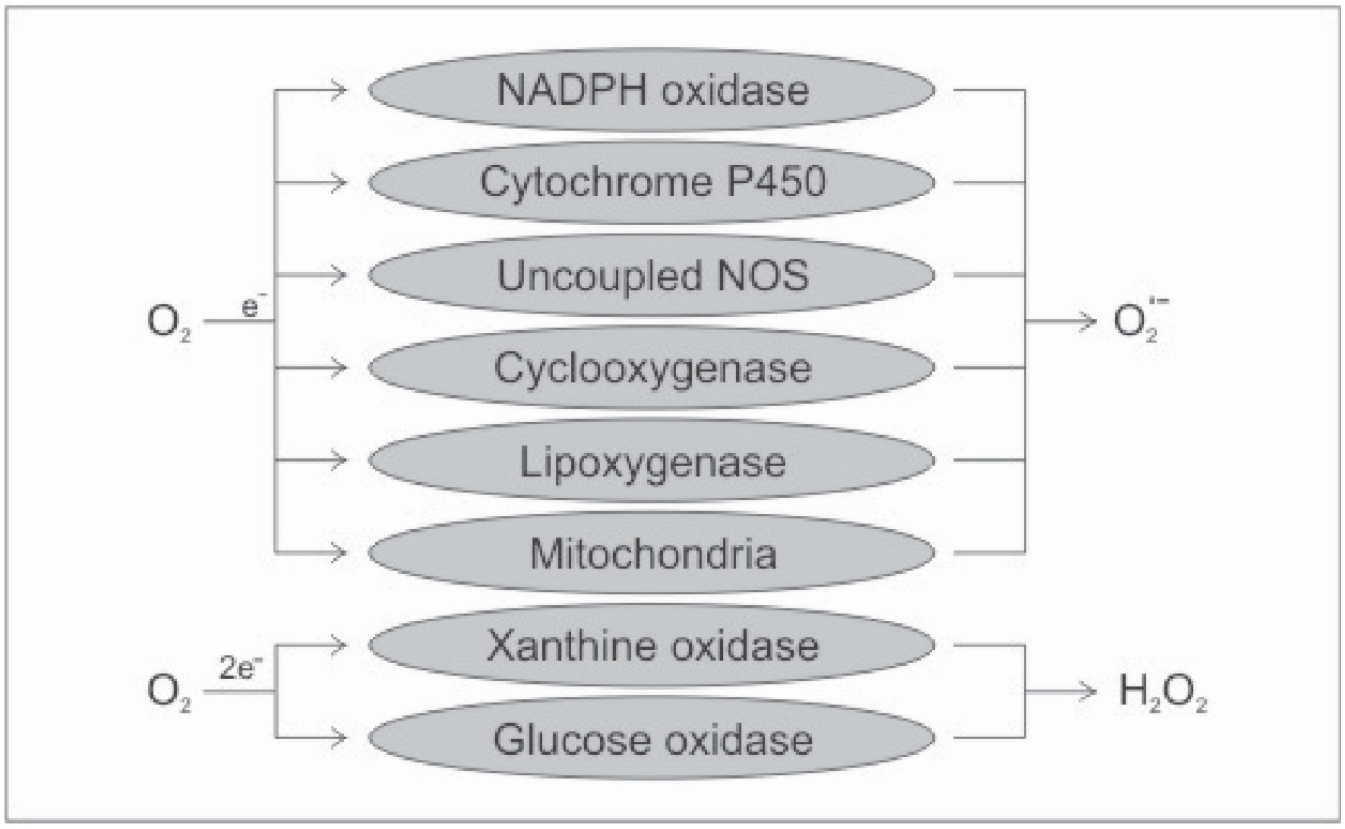


Fig. 2 : Sources of O_2^- and H_2O_2 in cells. The figure shows the enzymatic pathway of superoxide anion (O_2^-) and hydrogenperoxide (H_2O_2) generation in cells.

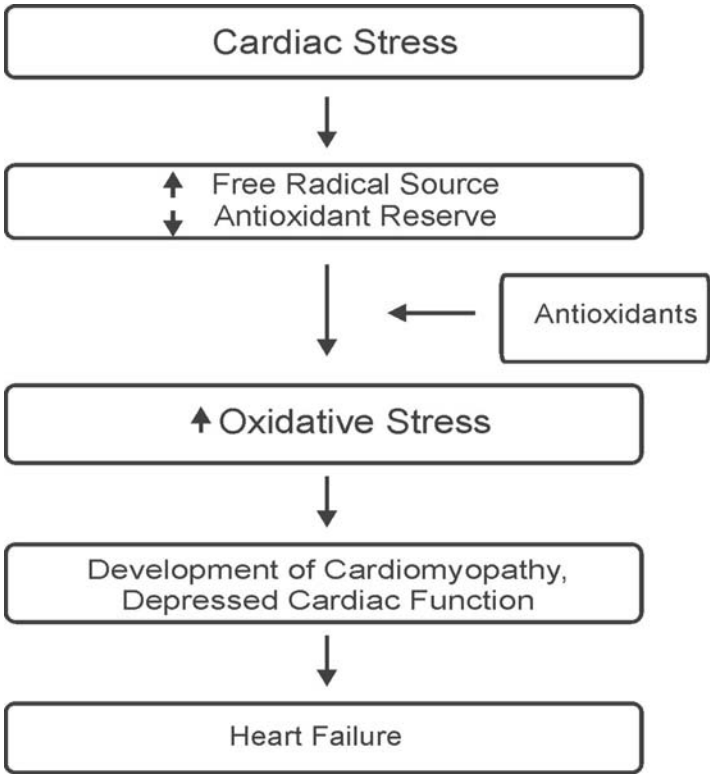
and progression of various cardiovascular diseases[28,29]. The free radicals are controlled by antioxidants levels and excessive free radical formation and insufficient removal by antioxidants leads to oxidative stress (OS) on heart[27]. The risk factors due to excess free radicals are use of tobacco, smoking, alcohol drinking, diet, pollution, heavy exercises and metabolic abnormalities lead to increased oxidative stress to heart[12]. The ROS can stimulate oxidation of LDL, low density lipoprotein, cholesterol, cholesterol derived species and modification of proteins which leads to foam cell formation and atherosclerotic plaques in arteries[5]. There is good evidence that vitamins C, E and ascorbic acid, tocopherols, exert a protective effect on the heart against CVD by reducing oxidative stress (OS).

The present status of antioxidants in human CVD protection is that the use of vitamins is necessary which regulates endothelial nitric oxide levels as well as by inhibiting cardiovascular inflammation, lipid peroxidation, platelet aggregation and LDL oxidation and to prevent endothelial dysfunction. The antioxidants also influence plaque stability. The antioxidants vitamins can reverse endothelial dysfunction induced by methionine and can restore endothelial function in hyperlipidemia children and young smokers. In patients of chronic heart failure, allopurinol, xanthine oxidase inhibitor, a potential antioxidant which reverses endothelial dysfunction in heavy smokers, type 2 diabetes and mild hypertension. The antioxidants slow down the thickening of arteries, atherosclerosis, and progression in CHD. It is reported that increased glutathione-1 peroxidase activity lowers the risk of CVD. The catalase enzyme inactivates ROS, superoxide dismutase enzyme by regulating the availability of nitric oxide and selenium by increasing glutathione peroxidase activity and protects CVD[42]. The natural antioxidants present in fruits and vegetables are flavonoids and phenolic compounds which protect heart from cardiovascular diseases[21,26]. The dietary factors based on cereals, pulses, spices, green vegetables, citrus fruits, palm and soybean oil, cod liver oil, sprouts, green peppers, whole grains, honey, walnuts and tea can significantly increase the liver antioxidants enzymes and their supplementation which reduces the risk of coronary heart disease (CHD).

Conclusions

Although there are many gaps in our understanding of the role of free radicals in the pathogenesis of cardiomyopathies and heart failure, based on the available data, some suggestions can be made (Fig. 1). Any acute

or chronic cardiac stress conditions, resulting in a relative deficit in the myocardial 'antioxidant reserve', are associated with an increase in myocardial 'oxidative stress'. The latter is capable of causing subcellular abnormalities, through mechanisms that are as yet poorly understood, that may lead to cardiomyopathic changes, depressed contractile function and heart failure. In this regard, the occurrence and importance of free radicals in cardiac pathophysiological conditions is now well established. Furthermore, the available evidence from animal and human studies illustrates that different antioxidants constituting an antioxidant reserve offer protection against oxidative stress-mediated myocardial changes. An understanding of the molecular basis of antioxidant changes will help to develop newer therapies for modulating the pathogenesis of heart failure.



References

1. Akasaki T, Ohya Y, Kuroda J, *et al*. "Increased expression of gp91phox homologues of NAD(P)H oxidase in the aortic media during chronic hypertension: Involvement of the renin-angiotensin system," *Hypertension Research*. 2006; **29** (10) : 813–820.
2. Bahorun T, Soobrattee MA, Luximon-Ramma V, Aruoma OI. Free radicals and antioxidants in cardiovascular health and disease. *Internet J. Med. Update*. 2006; **1** : 1–17.
3. Bayir H, "Reactive oxygen species," *Critical Care Medicine.* 2005; **33** (12) : S498–S501,.
4. Bedard K, Krause KH. "The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology," *Physiological Reviews.* 2007; **87** (1) : 245–313.
5. Boullier A, Bird DA, Chang MK, Dennis EA, Friedman P, Gillotre-Taylor K, Hörkkö S, Palinski W, Quehenberger O, Shaw P, Stein-Berg D, Terpstra V, Witztum JL. Scavenger receptors, oxidized LDL, and atherosclerosis. *Ann N Y Acad Sci.* 2001; **947** : 214-222; discussion 222-223.
6. Chatterjee M, Saluja R, Kanneganti S, *et al.* Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats. *Cell Mol. Biol.* 2007; **53** : 84–93.
7. Coyle CH, Martinez LJ, Coleman MC, Spitz DR, Weintraub NL, Kader KN. "Mechanisms of H2O2-induced oxidative stress in endothelial cells," *Free Radical Biology and Medicine.* 2006; **40** (12) : 2206–2213,
8. Droge W. Free radicals in the physiological control of cell function. *Review. Physiol. Rev.* 2002; **82** : 47–95.
9. Frei B. Reactive oxygen species and antioxidant vitamins. Linus Pauling Institute. Oregon State University. 1997.
10. Genestra M. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Review. Cell Signal.* 2007; **19** : 1807–1819.
11. Gordillo G, Fang H, Park H, Roy S. "Nox-4-dependent nuclear H2O2 drives DNA oxidation resulting in 8-OHdG as urinary biomarker and hemangioendothelioma formation," *Antioxidants and Redox Signaling.* 2010; **12**(8) : 933–943.
12. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 2nd ed. Clarendo Press, Ox- ford, UK, 1989.
13. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th. Oxford, UK: Clarendon Press; 2007.

14. Halliwell B. Biochemistry of oxidative stress. *Biochem. Soc. Trans.* 2007; **35** : 1147–1150.

15. Handy DE, Loscalzo J. "Redox regulation of mitochondrial function," *Antioxidants & Redox Signaling.* 2012; **16** (11) : 1323–1367.

16. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. "Role of oxidative stress in atherosclerosis," *The American Journal of Cardiology.* 2003; **91** (3) : 7–11.

17. Harrison DG. "Cellular and molecular mechanisms of endothelial cell dysfunction," *The Journal of Clinical Investigation.* 1997; **100** (9) : 2153–2157.

18. He F, Zuo L. "Redox roles of reactive oxygen species in cardiovascular diseases," *International Journal of Molecular Sciences.* 2015; **16** (12) : 27770–27780.

19. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. "Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease," *Circulation.* 2001; **104** (22) : 2673–2678.

20. Huang X, Zhang J, Liu J, *et al*. "Creactive protein promotes adhesion of monocytes to endothelial cells *via* NADPH oxidase-mediated oxidative stress," *Journal of Cellular Biochemistry.* 2012; **113** (3) : 857–867.

21. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardio-vasc Diabetol.* 2005; **4** : 5.

22. Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J. "NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart," *Proceedings of the National Academy of Sciences of the United States of America.* 2010; **107** (35) : 15565–15570.

23. Lavoisier AL. Recherches de M. Priestly surles differentes especes d'air. Opuscules Physiques et Chimiques, 1773, Chap XV. Reprinted in Oeuvres De Lavoisier. Paris: Imprimerie Imperiale, 1864.

24. Lavoisier AL. Alterations qu'eprouve l'air resire. Recueil des memoires de Lavoisier. 1785, Read to the Societe de Medicine. Reprinted as part of 'Memoires sur la respiration et al transpiration des animaux' in 'Les maitres de la pensee scientifique.' Paris: Gauthier-Villaus et cie, 1920.

25. Lee HH, Paudel KR, Kim DW. "*Terminalia chebula fructus* inhibits migration and proliferation of vascular smooth muscle cells and production of inflammatory mediators in RAW 264.7," Evidence-Based Complementary and Alternative Medicine, 2015; **2015**, Article ID 502182 : 10 pages.

26. Mahajan A, Tandon VR. Role of antioxidants and oxidative stress in cardiovascular diseases. *J Ind Rheumotol Assoc.* 2004; **12** : 39-42.

27. Maritim AC, Sanders RA, Watkins JB 3RD. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol.* 2003; **17** : 24-38.

28. Marx JL. Oxygen free radicals linked to many diseases. *Science.* 1987; **235** : 529-531.

29. Mayers ML. Enhancement of recovery of myocardial function. *Circulation.* 1985; **72** : 915-921.

30. McCord JM Fridovich I Superoxide dismutase. An enzymatic function for erythrocuprein (hemecuprein) *J Biol Chem* 1969; **244** : 6049-6055

31. Murphy MP. "How mitochondria produce reactive oxygen species," *Biochemical Journal.* 2009; **417** (1) : 1–13.

32. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.* 2007; **87** : 315–424.

33. Panth N, Park SH, Kim HJ, Kim DH, Oak MH. "Protective effect of Salicornia europaea extracts on high salt intake-induced vascular dysfunction and hypertension," *International Journal of Molecular Sciences.* 2016; **17** (7) : 1176.

34. Parthasarathy S, Santanam N, Ramachandran S, Meilhac O. Oxidants and antioxidants in atherogenesis: an appraisal. *J. Lipid Res.* 1999; **40** : 2143–2157.

35. Paravicini TM, Touyz RM. "NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities," *Diabetes Care.* 2008, **31** (2) : S170–S180,.

36. Paudel KR, Panth N, Kim DW, "Circulating endothelial microparticles: a key hallmark of atherosclerosis progression," *Scientißca.* 2016; **2016**, Article ID 8514056 : 9 pages.

37. Priestly J. 'Experiments and observations on different kinds of air', Vol II, Sections III-V, 1775:29–203. Reprinted in 'The discovery of oxygen', Part 1, Alembic Club Reprint No. 7. Kent, London: Simpkin, Marshall, Hamilton,

1894.

38. Reid MB. “Redox modulation of skeletal muscle contraction: what we know and what we don’t,” *Journal of Applied Physiology.* 2001; **90** (2) : 724–731.
39. Scheele CW. Chemische abhandlung von der luft und dem Feuer, Upsala and Leipzig. 1777, Reprinted as ‘The discovery of oxygen’, Part 2. Alembic Club Reprint No. 8. London: Gurney and Jackson, 1923.
40. Scherz-Shouval R, Elazar Z. “ROS, mitochondria and the regulation of autophagy,” *Trends in Cell Biology.* 2007; **17** (9) : 422–427.
41. Subash VK, Saritha G, Fareedullah MD. The role of antioxidants and oxidative stress in cardiovascular disease. *Ann Biol Res.* 2010; **1** : 158- 173.
42. Tandon VR, Verma S, Singh JB, Mahajan A. Role of antioxidants and oxidative stress in cardiovascular diseases. *Drug Review, JK Science.* 2005; **7** : 61-63.
43. Taverne YJHJ, Bogers AJJC, Duncker DJ, Merkus D. “Reactive oxygen species and the cardiovascular system,” *Oxidative Medicine and Cellular Longevity.* 2013; 15.
44. Touyz RM, Briones AM, Sedeek M, Burger D, Montezano AC, “NOX isoforms and reactive oxygen species in vascular health,” *Molecular Interventions.* 2011; **11** (1) : 27–35.
45. Valko M, Izakovic M, Mazur M, Rhodes CJ, et al. Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell Biochem.* 2004; **266** : 37–56.
46. Valko M, Morris H, Cronin MTD. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* 2005; **12** : 1161–1208.
47. Valko M, Rhodes CJ, Moncol J, Izakovic M, *et al.* Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Mini-review. Chem. Biol. Interact.* 2006; **160** : 1–40.
48. Valko M, Leibfritz D, Moncola J, Cronin MD, *et al.* Free radicals and antioxidants in normal physiological functions and human disease. Review. *Int. J. Biochem. Cell Biol.* 2007; **39** : 44–84.
49. Vogiatzi G, Tousoulis D, Stefanadis C. “The role of oxidative stress in atherosclerosis,” *Hellenic Journal of Cardiology.* 2009; **50** (5) : 402–409.
50. Wattanapitayakul SK, Bauer JA. “Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications,” *Pharmacology and Therapeutics.* 2001; **89** (2) : 187–206,.
51. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic diseases. *Review. Crit. Rev. Food. Sci. Nutr.* 2004; **44** : 275–295.
52. Young I, Woodside J. Antioxidants in health and disease. *J. Clin. Pathol.* 2001; **54** : 176–186.
53. Zorov DB, Juhaszova M, Sollott SJ. “Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release,” *Physiological Reviews.* 2014; **94** (3) : 909–950.
54. Zuo L, Zhou LT, Pannell BK, Ziegler AC, Best TM. “Biological and physiological role of reactive oxygen species—the good, the bad and the ugly,” *Acta Physiologica.* 2015; **214** (3) : 329–348. s