Free Radicals and Antioxidants in Cardiovascular Health and Disease

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ABSTRACT: Current hypotheses favour the concept that lowering oxidative stress can have a health benefit. Free radicals can be overproduced or the natural antioxidant system defenses weakened, first resulting in oxidative stress, and then leading to oxidative injury and disease. Cardiovascular disease is one example of this process. This disorder continues to be the major cause of premature death worldwide. Oxidation of human low-density lipoproteins is considered an early step in the progression and eventual development of atherosclerosis, one of the leading causes to cardiovascular dysfunction. Compelling support for the involvement of free radicals in disease development originates from epidemiological studies showing that an enhanced antioxidant status is associated with reduced risk of several diseases. Dietary nutraceuticals such as vitamins C, E and polyphenolics and reduction of cardiovascular disease incidence are a notable example. This paper reviews the biology of ROS/RNS, their pathways through which they relate to the pathology of cardiovascular disease and discusses the putative roles that antioxidants, including phenolics, may play in controlling oxidative stress and reduce the incidence of cardiovascular disease.

KEY WORDS: Oxidative stress, cardiovascular disease, atherosclerosis, inflammation, cell signaling and transduction mechanisms, antioxidants, dietary phenolics.

INTRODUCTION: Cardiovascular disease (CVD) is the leading cause of death in the United States, Europe and Japan[1] and is poised to become the most significant health problem worldwide. According to the World Health Organisation (WHO), an estimated 17 million people die of CVDs, particularly heart attack, stroke and heart failure, every year. In Mauritius deaths due to cardiovascular dysfunctions have kept on increasing for the last 10 years attaining the 51% mark in 2004[2]. Cardiovascular disease is of multifactorial etiology associated generally to a variety of risk factors for its development including hypercholesterolaemia, hypertension, smoking, diabetes, poor diet, stress and physical inactivity amongst others. During the last few decades, research data has prompted a passionate debate as to whether oxidation, or specifically, oxidative stress mediated by free radicals/reactive oxygen species (ROS)/reactive nitrogen species (RNS), is a primary or secondary cause of many chronic diseases. As a result, scientific resources have focused to a large extent on the role that antioxidants could play to delay or prevent oxidative stress and consequently the incidence of chronic disorders. This article will review the biology of ROS/RNS, their pathways through which they relate to the pathology of cardiovascular disease. We shall also discuss the roles that antioxidants may play in controlling oxidative stress and reduce the incidence of CVDs.

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BASIC CONCEPTS OF FREE RADICALS, REACTIVE NITROGEN SPECIES, REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS:

A free radical is any chemical species (capable of independent existence) possessing one or more unpaired electrons, an unpaired electron being one that is alone in an atomic or molecular orbital. Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron (free radicals may also be formed by collision of the non radical species by a reaction between a radical and a molecule -which must then result in a radical since the total number of electrons is odd), by cleavage of a radical to give another radical and, finally via redox reactions [3,4,5]. Radicals are generally less stable than non-radical species, although their reactivity varies **(Table 1)**.

Free radicals and reactive oxygen/nitrogen species of importance in living organisms include hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot}$) and peroxyl ($ROO^{\cdot}$). Peroxynitrite ($OONO^-$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), nitrous acid (HNO_2) and dinitrogen trioxide (N_2O_3) are not free radicals but can easily lead to free radical reactions in living organisms. The term 'reactive oxygen species' (ROS) and 'reactive nitrogen species' (RNS) is a collective term that includes not only the radicals but also the non-radicals. Oxidative stress is the term referring to the imbalance between generation of reactive oxygen species and the activity of the antioxidant defences.

Humans and other aerobes are able to tolerate oxygen (O_2) because, at the same time that organisms were evolving electron-transport chains and other enzyme systems to utilize this molecule, antioxidant defenses to protect against the toxic effects of O_2 were evolving in parallel. The aerobic life-style offers great advantages, but is fraught with danger. ROS/RNS are constantly generated in vivo via two main types of processes. ROS/RNS can arise from accidental generation; this encompasses such mechanisms as 'leakage' of electrons onto O_2 from mitochondrial electron transport chains; nuclear membrane, endoplasmic reticulum (xenobiotic metabolism, prostaglandin synthesis) and hepatocytes (detoxification) contain electron transport systems, cytochrome P-450 and b_5, which produce free radicals**[6].** Accidental generation also includes the direct reaction of autoxidisable molecules with molecular O_2, generating superoxide free radical. The major biological process leading to $O_2^{\cdot-}$ generation is the electron transport associated with mitochondrial membrane; ubiquinone-cytochrome *b* is the most important site of $O_2^{\cdot-}$ production. It has been estimated that about 1-3% of O_2 respired is converted to $O_2^{\cdot-}$, a rate that increases during periods of increased energy metabolism. $O_2^{\cdot-}$ also is produced by phagocytic cells (neutrophils, monocytes, macrophages, eosinophils) and helps them to inactivate viruses and bacteria. When these cells encounter a phagocytable particle, their O_2 consumption increases tremendously ('respiratory burst') with the activation of a membrane-located enzyme (NADPH-oxidase) which catalyse the reduction of O_2 into $O_2^{\cdot-}$. $O_2^{\cdot-}$ participate in the production of very reactive chemical species such as $OH^{\cdot}$, hypochlorite and chloramines **(Figure 1)**. The importance of ROS production by the immune system is clearly exemplified by patients with granulomatous disease**[7].** These patients have defective membrane-bound NADPH oxidase system thus cannot produce $O_2^{\cdot-}$, resulting in multiple and persistent infection, especially *Staphylococcus aureus*. $O_2^{\cdot-}$ is also generated by a variety of cytosolic and membranes-bound enzymes, including xanthine oxidase, cytochrome 450 complex and phospholipase A_2.

Many biomolecules undergo autooxidation reaction on contact with O_2 producing ROS, for example catecholamines, tetrahydrofolates and reduced reduced flavins react directly with O_2 to form $O_2^{\cdot-}$**[8].** Several sugars, including glucose, react with proteins to produce oxygen radicals. It has been suggested that years of exposure of body tissues to elevated blood glucose in diabetic patients can result in them suffering an 'oxidative stress' that may contribute to the side effects of hyperglycaemia [9].

H_2O_2 is a nonradical. It resembles water in its molecular structure and is very diffusible within and between cells. H_2O_2 is able to diffuse across biological membranes, whereas $O_2^{\cdot-}$ does not. As well as arising from dismutation of $O_2^{\cdot-}$, H_2O_2 is produced by the action of several oxidase enzymes *in vivo*, including amino acid oxidases and the enzyme xanthine oxidases [10]. Xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine, and of xanthine to uric acid; oxygen is simultaneously reduced both to $O_2^{\cdot-}$ and to H_2O_2. Xanthine oxidase is present in many mammalian tissues, especially in the gastrointestinal tract [11]. Phagocytic cells generate substantial amount of H_2O_2 that is responsible

for the cytotoxic action observed in localized tissue inflammation. Peroxisomes contain several ROS generating enzymes including glucose oxidase, amino acid oxidase, xanthine oxidase, glycollate and urate oxidase, as well as flavoprotein oxidases [12]**.** The major by-product of these oxidases is H_2O_2, thus explaining the high level of catalase present in peroxisomes which detoxifies H_2O_2 to H_2O. Much of the toxicity of O_2^{-} and H_2O_2 involves formation of $OH^{\cdot}$ which is the most reactive free radical in vivo with an estimated half-life of about 10^{-9} sec In the presence of H_2O_2, superoxide act as the precursor of hydroxyl radical ($OH^{\cdot}$) **(Figure 1)**. This reaction is very slow and cannot occur in the absence of catalyst; iron ion acts as catalyst for this reaction. The superoxide anion reduces Fe^{3+} to Fe^{2+}. In the presence of Fe^{2+}, H_2O_2 readily decompose into $OH^{\cdot}$ and OH^{-}. This reaction is known as the *Fenton reaction* [13]**.** Thus, the simultaneous presence of superoxide anion, H_2O_2, and iron ion lead to the production of hydroxyl radical. Copper ions also react with H_2O_2 to form $OH^{\cdot}$. Hydroxyl radical may also be formed by exposure of living organisms to ionising radiation which causes fission of O-H bonds in water, to give $H^{\cdot}$ and $OH^{\cdot}$ [14].

The bioavailability of metal ions is strictly control under normal physiological condition. After absorption from the gut, metal ions are complexed to transport proteins (transferrin, ceruloplasmin). Excess metal ions are stored coupled to storage proteins such as ferritin, haemosiderin. Hydroxyl radical generation can take place when the homeostasis is altered. For example, tissue injury may cause the release of metal ions from damaged cells, contributing to a worsening of the injury. Hydroxyl radical reacts at a diffusion-controlled rate with almost all molecules in living cells. Hence, when hydroxyl radical is formed *in vivo*, it damages whatever it is generated next to, as hydroxyl radical cannot migrate any significant distance within the cell. $OH^{\cdot}$ attacks proteins, DNA, polyunsaturated fatty acid (PUFA) and many other biological molecules.

HOCl is produced by the neutrophil-derived enzyme myeloperoxidase at sites of inflammation and when activated neutrophils infiltrate reoxygenated tissue[15]. The enzyme oxidizes chloride ions in the presence of hydrogen peroxide **(Figure 1)**. HOCl is not a free radical, but it is a potent chlorinating and oxidizing agent. HOCl reaction with cholesterol causes chlorohydrins that could disrupt cell membranes, leading to cell lysis and death. On the basis of this observation, the cholesterol chlorohydrins have been suggested to be potential biomarkers for oxidative damage associated with neutrophil/monocyte activation[16]. HOCl can attack many other biological molecules. Thiols and thioethers are particularly reactive and other compounds, including ascorbate, urate, pyridine nucleotides and tryptophan are oxidized by HOCl. The main biological chlorination reactions are with amine groups to give chloramines; with tyrosyl residues to give ring chlorinated products; with unsaturated lipids to give chlorohydrins and ring chlorination of cytosine resides in nucleic acids [17].

$NO^{\cdot}$ is formed from the oxidation of L-arginine by nitric oxide synthase (NOS) of which three isoforms are known. $NO^{\cdot}$ has a variety of functions, including memory formation, synaptic plasticity and synaptogenesis. It is thought that the endothelium derived-relaxing factor (EDRF) produced by vascular endothelium, which is an important mediator of vascular responses induced by several pharmacological agents (including brandykinin) is identical to $NO^{\cdot}$ [18,19].

Excess $NO^{\cdot}$ is cytotxic, both directly (e.g. by combining with tyrosine) and indirectly, by forming $ONOO^{-}$. Since $NO^{\cdot}$ relaxes smooth muscle in blood vessel walls resulting in lower blood pressure, O_2^{-} by removing $NO^{\cdot}$ can be a vasoconstrictor. Thus, excess vascular O_2^{-} production could contribute to hypertension and vasospasm[20]**.** $ONOO^{-}$ formed in blood vessel walls may aggravate atherosclerosis by depleting antioxidants and causing peroxidation of LDL. Furthermore, nitration of tyrosine by $ONOO^{-}$ may interfere with cell signal transduction [21]. A role for $NO^{\cdot}$ has also been demonstrated in such human diseases as malaria where $NO^{\cdot}$ appears to be partly involved in resistance to malarial infection, in cardiovascular disease, acute inflammation, cancer, neurodegenerative diseases, and diabetes. Moreover $NO^{\cdot}$ has been implicated in adult respiratory distress syndrome, septic shock, hypertension, thrombosis, renal failure, AIDS encephalopathy, bronchospasm, stroke and male impotence [22].

Table 1 Chemical Reactions of Free Radicals*

Unimolecular Radical Reactions

Reactions result from the instability of the first formed radical. The radicals may completely decompose or rearrange before reaction with other molecules or radicals present.

***Decomposition*:** reaction in which the radical decomposes to give a stable molecule and a new radical

***Rearrangement*:** 1. breaking of an adjacent C-C bond in a cyclic system with concomitant formation of a new bond, usually carbonyl and a new isomeric radical
2. migration of an atom, via intramolecular abstraction by the radical center, thus creating a new, isomeric radical.

Radical-Molecule Interactions

***Addition to unsaturated systems*:**

1. Addition of a radical to an olefinic double bond to give a new saturated, radical. Typical reaction is the radical induced polymerization of olefins.
2. Addition of a radical to an aromatic double bond. This intermediate step is widespread in free radical chemistry, e.g. in the radical substitution of aromatic compounds (homolytic aromatic substitution). The net overall reaction is displacement of an aromatic substituent by a radical:

$$AR — X + Y^{\cdot} \rightarrow AR — Y + X^{\cdot}$$

***Abstraction or displacement: S_H2 reactions*†**

– Biomolecular reaction involving homolytic attack of a radical on a molecule. The radical attacks a univalent atom, usually a terminal halogen or hydrogen in an abstraction reaction to give rise to a new radical, e.g.

$$Ph^{\cdot} + CBrCl_3 \rightarrow {}^{\cdot}CCl_3 + PhBr$$

– Homolytic substitution at multivalent atoms also occurs but both do not normally occur at saturated carbon centers.

Reaction with oxidizing agents

Radicals readily undergo 1-electron oxidations with oxidizing reagents of suitable redox potential to give positive ions. Example is the Meerwein reaction, which involves the oxidation of cinnamyl derived radicals by cupric ions:

$$Ph\dot{C}HCHRCO_2\,Et + Cu^{2+} \rightarrow PhC^{+}HCHRCO_2Et + Cu^{+}$$

Radical-Radical Interactions

Dimerization or radical coupling

Localized radicals (methyl, phenyl radicals) react readily with little chance of dimerization. Only delocalized radicals have a high probability of dimerization in solution. Thus,

$$R'^{\cdot} + R''^{\cdot} \rightarrow R' - R''$$

When R' = R", the reaction is dimerization and when R' ≠ R" the reaction is radical coupling or combination.

Radical disproportionation

Involves collision of the radicals resulting in the abstraction of an atom, usually hydrogen, by one radical from the other. This leads to the formation of two stable molecules, with the atom abstracted being β to the radical center:‡ e.g. the disproportionation of two phenylethyl radicals to give styrene and ethylbenzene.

* The reader is referred to Moad and Solomon [5] for an extensive overview of the reaction sequences highlighted in this table and an examination of the complex chemistry of organic radicals.

† S_H2 stands for substitution homolytic biomolecular.

‡ The disproportionation reaction derives its driving force from the formation of two newstrong bonds and from the fact that the β-CH bonds in radicals are usually weak.

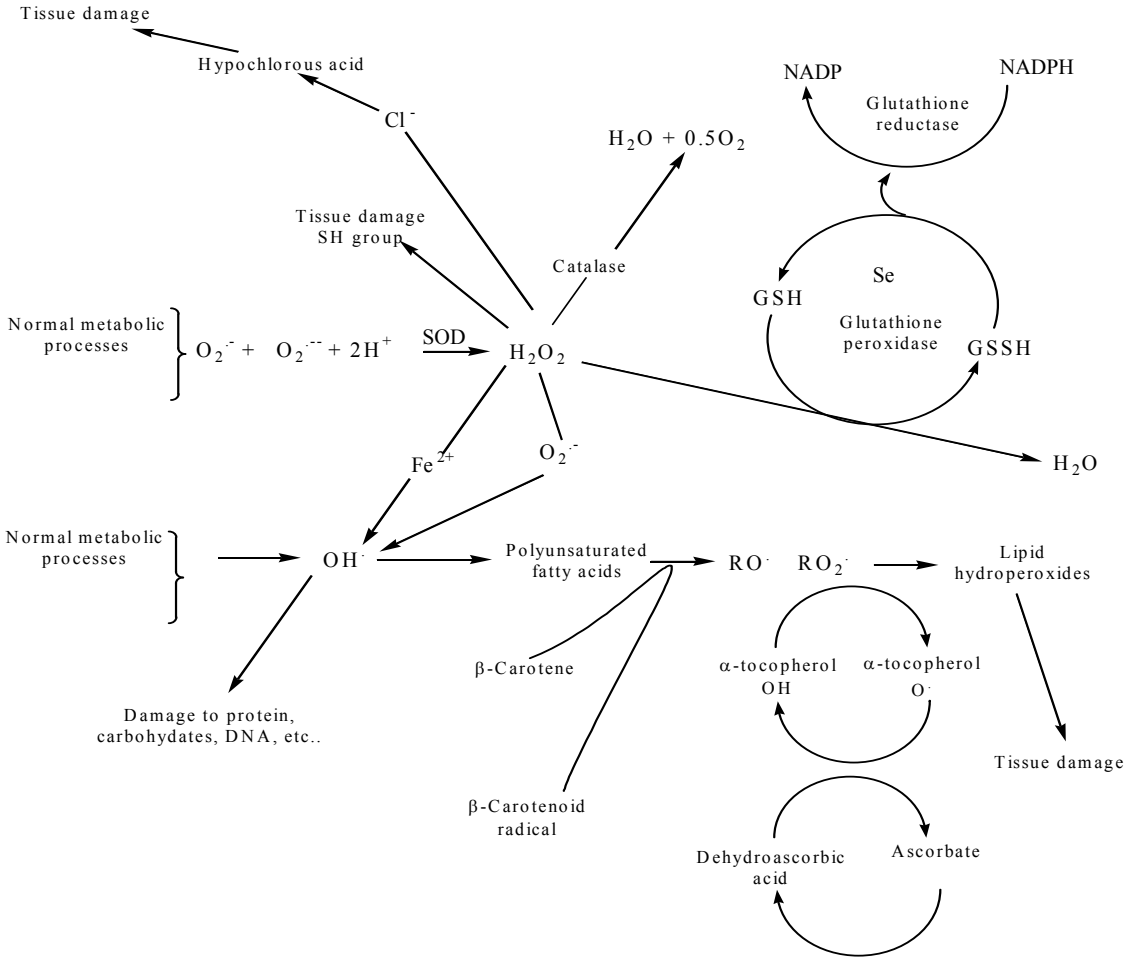

Figure 1: Inter-relationship between reactive oxygen species and antioxidants.

REACTIVE OXYGEN SPECIES AND THE CARDIOVASCULAR SYSTEM:

Reactive oxygen species have been considered deleterious to cell function and there is good evidence to suggest that they play a role in the pathophysiology of cardiac disease states. However, direct cause and effect relationships have not been clearly delineated. The increase in the generation of ROS under several pathophysiological conditions, that seem to be related to inflammatory processes, is still to be comprehensively understood; this may be due to difficulties in defining their site of origin. Impaired mitochondrial reduction of molecular oxygen may be an intracellular source. Secretions by phagocytic white blood cells, dysfunctional endothelial cells, or the auto-oxidation of catecholamines may be the extracellular sources. ROS may also result from cellular injury due to exposure to ionizing radiation, ultraviolet rays, cigarette smoking or other air pollutants. Besides their deleterious effects, ROS are also now being recognized as important regulators of cell function and modulators of cell signalling pathways.

ROS in pathophysiology of Heart disease:

One of the strategies used to assess the role of oxidative stress in the pathogenesis of cardiac dysfunction has been to expose isolated cardiac tissues to a defined oxidation stress condition and study the resulting effects [23-31]. Further in vivo and ex vivo studies have provided precious evidence supporting the role of oxidative stress in a number of conditions (atherosclerosis, ischemia-reperfusion injury, hypertension, catecholamine-induced cardiomyopathy, diabetic cardiomyopathy, cardiac hypertrophy and congestive heart failure etc...) leading to severe cardiovascular dysfunctions. In this review the

role of ROS in atherosclerosis is being emphasized as, besides being considered as the major cause of morbidity and mortality[32] its outcome is also linked to other conditions leading to cardiovascular disorders. The role of ROS in other above-mentioned conditions has been extensively reviewed and the reader is referred to a number of excellent reports[33, 34].

Most cardiovascular events are secondary to atherosclerosis, a disease of the arteries involving a local thickening of the vessel wall.

A stroke or myocardial infarction occurs when the lumen of the vessel becomes completely occluded, usually by a thrombus forming at the site of a plaque. Atherosclerotic lesions are thought to be initiated by emigration of monocytes into the arterial inner core (tunica intima), recruited by adhesion molecules, possibly in response to arterial endothelium injury[35]. A variety of factors have been implicated in causing this initial injury, including mechanical damage from flow stress worsened by high blood pressure, viral infection (herpes viruses and cytomegalovirus), exposure to blood-borne toxins such as xenobiotics from cigarette smoke and elevated levels of normal metabolites, such as glucose, homocysteine or cholesterol[36]. Although a high level of plasma cholesterol is considered to trigger atherosclerosis, the oxidation of cholesterol seems to be a necessary step. In fact, uptake of oxidized low-density lipoprotein ($_{ox}$LDL) was shown to be an early event leading to the development of atherosclerosis **(Figure 2)**. $_{ox}$LDL and oxidized lipoproteins have been reported to stimulate $O_2^{\bullet-}$ formation leading to apoptosis of cells in the umbilical vascular wall; this was prevented by treatment with antioxidants SOD and catalase[37]. In cultured human coronary artery smooth muscle cells, low levels of $_{ox}$LDL stimulate the extracellular matrix synthesis indicating the involvement of oxidative stress in the pathogenesis of atherosclerosis[38]. High levels of $_{ox}$LDL were apoptotic implicating the additive role of ROS in increased plaque vulnerability; this effect was reduced by probucol and catalase [38]. Patients with atherosclerosis and hypercholesterolemia showed higher susceptibility of LDL to oxidation in comparison to patients treated with lipid-lowering agents such as lovastatin and probucol[39].

In the atherosclerotic lesion produced in the rabbit aorta, significant increases in the iron content were observed suggesting that iron-catalysed free radical reactions may be associated with the development of atherosclerosis[40]. The occurrence of intracellular Ca^{2+}-overload has been proposed as a mechanism of injury due to oxidative stress because human endothelial cells subjected to oxidative stress showed an increase in the level of intracellular Ca^{2+} and plasma membrane blebbing[41]. Endothelial dysfunction may play an important role in the atherosclerotic process because in patients with atherosclerosis, the antioxidants, probucol and ascorbic acid, improved the endothelium-dependent relaxation suggesting the involvement of ROS in endothelial dysfunction[42]. Increased production of $O_2^{\bullet-}$ has been implicated in the impaired endothelium-dependent relaxation in cholesterol fed rabbits and was suggested to be an early event in the hypercholesterolemic atherosclerotic process[43]. Oxidative inactivation of $NO^{\bullet}$ by superoxide has been proposed as a plausible explanation for endothelial dysfunction[44]. When exposed together, $O_2^{\bullet-}$ and $NO^{\bullet}$ react with each other three times faster than the reaction rate of $O_2^{\bullet-}$ with either Mn^{2+} and Cu^{2+}/Zn^{2+}-SOD[45]. Therefore, $O_2^{\bullet-}$ would preferentially react with $NO^{\bullet}$ rather than SOD and cause inactivation of NOd. In human atherosclerotic arteries, the production of endothelial nitric oxide synthase (the enzyme catalysing $NO^{\bullet}$ formation) as well as $NO^{\bullet}$ has been shown to be depressed[46]. SOD was shown to protect the inactivation of $NO^{\bullet}$ in the canine coronary artery [47]. The generation of $O_2^{\bullet-}$ was thought to be due to the activation of the vascular and endothelial enzyme NADH/NADPH oxidase[42]. Moreover, an increase in NADH/NADPH oxidase-dependent vascular $O_2^{\bullet-}$ was reported in hypercholesterolaemic rabbits[48]. Oxidation of $NO^{\bullet}$ by $O_2^{\bullet-}$ results in the formation of peroxynitrite which could initiate lipid peroxidation or play a role in the oxidation of lipoproteins[49,50]. Both of the above may be important steps in the development of atherosclerosis.

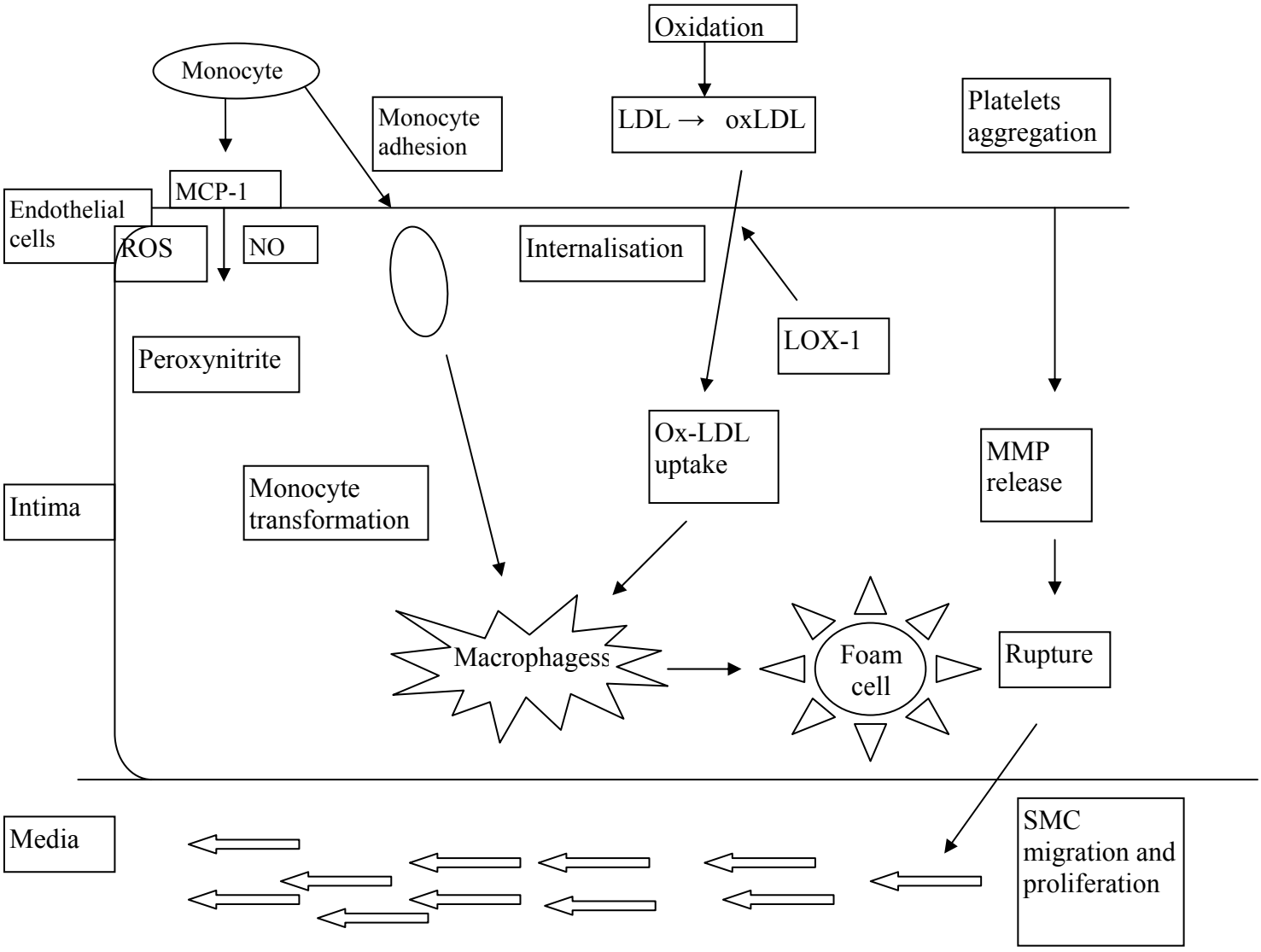

Figure 2: ROS and atherosclerosis: increased production of ROS may affect four fundamental mechanisms that contribute to atherosclerosis (i) oxidation of LDL to oxLDL; (ii) endothelial cell dysfunction; (iii) vascular smooth muscle cells migration and proliferation as well as MMPs release; (iv) monocyte adhesion and migration as well as foam cell development due to uptake of ox-LDL.

ROS in Mediated Signal Transduction Pathways in Cardiovascular disorders:

The cardiovascular system is a highly complex, well organised system in which signal transduction plays critical physiological and pathophysiological roles **(Figure 3)**. The cellular elements of the heart and vascular wall are equipped with an array of specific receptors and with complex intracellular machinery that facilitates and drives appropriate responses to extracellular stimuli. All vascular cell types, including endothelial cells, smooth muscle cells, adventitial fibroblasts, and resident macrophages, produce ROS[51-55]. Of particular importance in the vasculature are superoxide (O_2^{-}) and hydrogen peroxide (H_2O_2), since these ROS act as inter- and intra-cellular signaling molecules. The major source of ROS in the vascular wall is non-phagocytic NADPH oxidase, which is regulated by vasoactive agents

(Ang II, ET-1, thrombin, serotonin), cytokines (IL-1, TNFa), growth factors (PDGF, IGF-1, VEGF) and mechanical forces (cyclic stretch, laminar and oscillatory shear stress). High levels of low-density lipoprotein (LDL), especially in the form of oxidized low-density lipoprotein (ox-LDL), have also been shown to increase intracellular ROS generation. Under physiological conditions, vascular production of ROS and the consequent activation of redox-dependent signaling pathways and induction of redox-sensitive genes are tightly regulated. However, in pathological conditions, such as in hypertension, atherosclerosis, hyperlipidemia, hyperhomo-cysteinemia, and diabetes, where generation of ROS is increased and the renin angiotensin system may be upregulated, these redox-sensitive events may contribute to cellular processes involved in vascular dysfunction and structural remodeling [56-58].

Redox signalling has been suggested in vascular smooth muscle proliferation, atherosclerosis, angiogenesis, cardiac hypertrophy, fibrosis[59]. Modulation of intracellular signaling pathways (MAPKs), and the subsequent activation of downstream redox sensitive transcription factors like NF-κB, HIF-1, AP-1 results in alterations in gene and protein expression[60] that significantly enhance cardiac dysfunction. Increased bioavailability of vascular ROS leads to VSMC growth, migration, collagen deposition, and altered MMP activity, important factors in arterial remodeling in cardiovascular disease [56-58]. In endothelial cells, oxidative excess induces apoptosis and aniokis (cell shedding), leading to endothelial cell loss and resultant impaired endothelial function. In addition, oxidative stress stimulates activation of transcription factors (e.g., NFkB and AP-1) and pro-inflammatory genes (cytokines, interleukins), upregulation of adhesion molecules (e.g., ICAM, VCAM, PECAM), stimulation of chemokine production (e.g., MCP-1) and recruitment of inflammatory cells (monocytes, macrophages), critical processes involved in vascular inflammation and injury[61]. Increased vascular $^{\cdot}O_2^-$ and H_2O_2 also impair endothelium-dependent relaxation, increase contractile reactivity and alter vascular tone. These effects may be mediated directly by elevating cytosolic Ca^{2+} concentration or indirectly by reducing concentrations of the vasodilator $NO^{\cdot}$. A common link between free radicals and the aforementioned pathological condition is the disrupted intracellular signal transduction networks and this suggests a rationale for targeting these pathways in chemoprevention.

ANTIOXIDANTS AND THEIR RELEVANCE TO CARDIOVASCULAR DISEASE:

An antioxidant has been defined as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate"[38]. When ROS/RNS are generated in vivo, their actions are opposed by intricate and coordinated antioxidant lines of defence systems[61]. These include enzymatic and non-enzymatic antioxidants that keep in check ROS/RNS level and repair oxidative cellular damage **(Figure1)**. The major enzymes, constituting the first line of defence, directly involved in the neutralization of ROS/RNS are: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) **(Figure 1)**[61]. SOD is a cytoplasmic and mitochondrial enzyme, which accelerate the dismutation of superoxide. There are three forms of SOD: an extracellular and an intracellular copper/zinc (Cu/Zn) and a mitochondrial, manganese (Mn) SOD. All three forms catalyse the dismutation of O_2^- to H_2O_2. Because SOD enzymes generate H_2O_2, they work in collaboration with H_2O_2-removing enzymes. CAT, an exclusively peroxisomal enzyme in most tissues, converts H_2O_2 to water and O_2. However, the most important H_2O_2-removing enzymes are the selenoprotein GPx enzymes. GPx enzymes remove H_2O_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power **(Figure 3)**. Glutathione peroxidase also catalyse the reduction of unstable hydroperoxides at the expense of GSH [62].

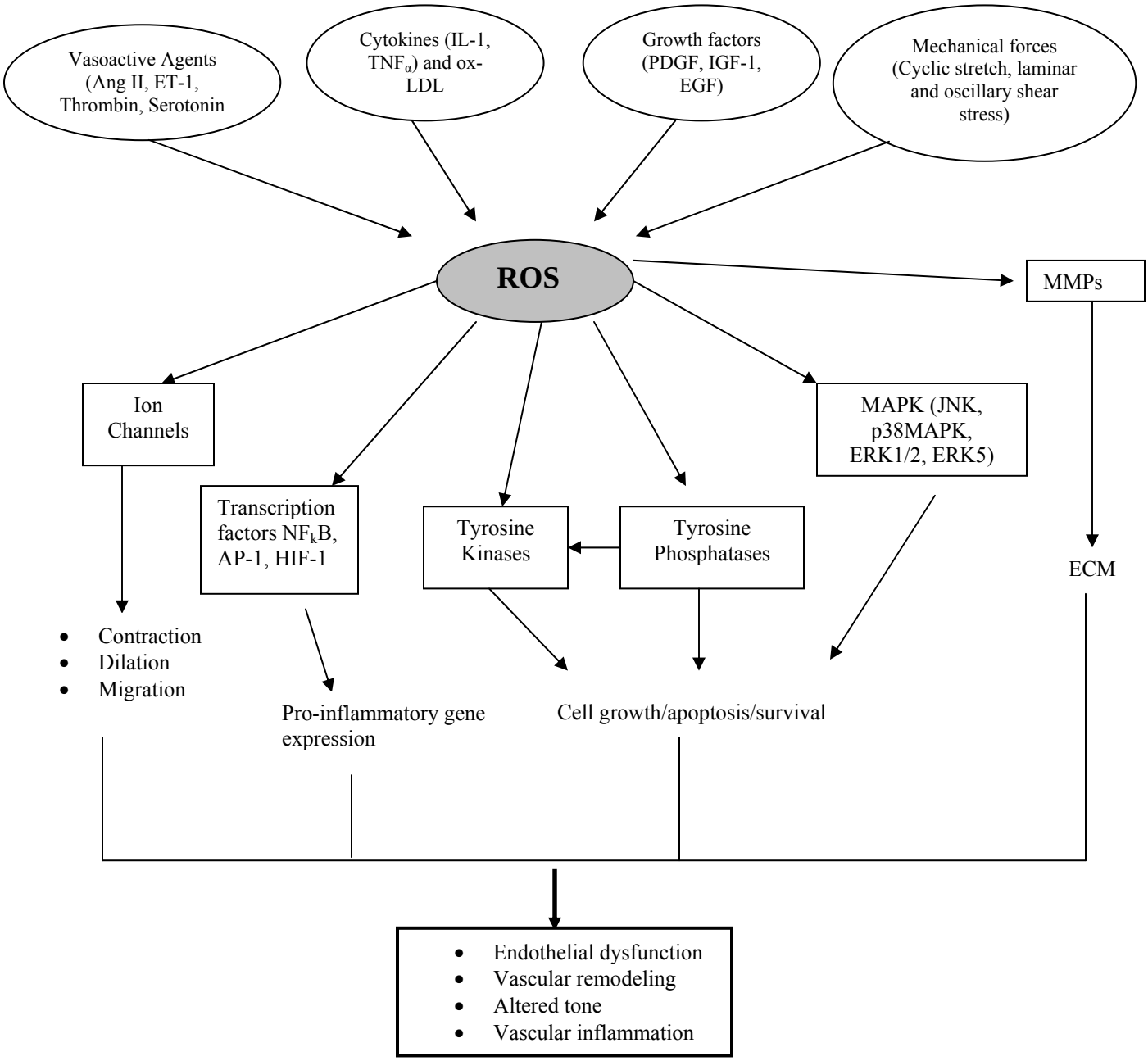

Figure 3: Redox-dependent signaling pathways in vascular smooth muscle cells: The increase ROS which is produced from NSDPH oxidase may modify the activity of tyrosine kinases, such as Src, Ras, JAK2, Pyk2, PI3K, and EGFR, as well as mitogen-activated protein kinases (MAPK), particularly p38MAPK, JNK and ERK5. ROS may inhibit protein tyrosine phosphatase activity, further contributing to protein tyrosine kinase activation. ROS also influence gene and protein expression by activating transcription factors, such as NF□B activator protein-1 (AP-1) and hypoxia-inducible factor-1 (HIF-1). ROS stimulate ion channels, such as plasma membrane Ca^{2+} and K^{+} channels, leading to changes in cation concentration. Activation of these redox-sensitive pathways results in numerous cellular responses which, if uncontrolled, could contribute to hypertensive vascular damage.

The second line of defence is represented by radical scavenging antioxidants such as vitamin C, vitamin A and plant phytochemicals like phenolics (emphasised later in this review) that inhibit the oxidation chain initiation and prevent chain propagation [63]. This may also include the termination of a chain by the reaction of two radicals. The repair and de novo enzymes act as the third line of defense by repairing damage and reconstituting membranes. These include lipases, proteases, DNA repair enzymes and transferases [64].

A number of studies have been conducted to explore the roles of various antioxidants in cardiovascular diseases. Diphenylene iodonium (DPI), a potent inhibitor of NADH/NADPH oxidase enzyme has been shown to suppress p38 MAP kinase-mediated VSMC hypertrophy *in vitro*[65]. Furthermore, it was reported that *N*-acetyl-*L*-cysteine (NAC), a radical scavenger and intracellular glutathione precursor, inhibited endothelin-induced ROS generation, JNK activation and VSMC proliferation[66]. Probucol is a modestly potent LDL-lowering agent with powerful anti-oxidant properties that effectively inhibits the oxidative modification of LDL, independent of its lipidlowering effect. By exerting anti-oxidant effect, it may inhibit VCAM-1 and MCP-1 expression and inhibit human aortic SMC proliferation as well as atherogenesis [67].

The HMG-CoA reductase inhibitors, also known as statins, are potent lipid-modifying agents. There is overwhelming evidence from clinical studies that reducing plasma LDL levels with statins, results in a markedly lower risk of cardiovascular events related to atherosclerosis[68]. Recent studies in patients with established CAD show that these agents can cause a modest regression of atherosclerotic lesions. It has been suggested that the antiatherosclerotic effect of statins may be independent of their LDL-lowering effect [69,70].

AT1R blockers and ACE inhibitors are widely used to treat patients with hypertension and/or congestive heart failure by blocking the effect of Ang II or its formation. Recent studies show that Ang II is also a strong stimulus for ROS generation[71-73] and AT1R blockers as well as ACE inhibitors inhibit the expression of pro-atherogenic factors by decreasing ROS production in vascular endothelial cells and in animal models[74-77]. A large number of studies have provided direct evidence showing the antiatherosclerotic effects of these agents[78-80]. Statins and AT1R blockers exert synergistic effects on the inhibition of atherosclerotic lesions in the apo E-knockout mice placed on a high cholesterol diet[81].

PPAR-γ ligands, which are widely used in the treatment of type II diabetes, have been identified as potent antioxidants[82]. By suppressing NADPH oxidase expression and reducing intracellular ROS production, these agents inhibit the expression of several proatherogenic proteins and apoptosis in vascular endothelial cells and SMCs[83,84]. Experimental studies show that thiazolidinedione, a potent PPAR-γ ligand, reduces the size and number of atherosclerotic lesions in the vessel wall by modulating foam cell formation and inflammatory responses of macrophages[85].

Vitamins E and C have been demonstrated to reduce the progression of atherosclerosis. Trolox C, a water-soluble vitamin E analogue, and vitamin C (ascorbic acid) abolished the stimulatory effect of Ang II on JNK and p38 activity in VSMC[86]. Intake of vitamin E decreased the incidence of cardiovascular events in the population of ischemic heart disease patients in the Cambridge Heart Antioxidant Study (CHAOS)[87]. Clinical studies show that while these anti-oxidant vitamins do not reduce endpoints related to atherosclerosis, they improve endothelial function by increasing local NO bioavailability and, therefore, endothelium-dependent vasodilation. It has been reported that high dose of vitamin C infusions improved endothelial dysfunction in patients with renovascular hypertension[88]. However, a GISSI-3 study[89] and HOPE study[90] could not show significant beneficial effects of vitamin E in the secondary prevention of coronary artery disease. A Heart Protection Study (HPS) in the UK also could not demonstrate any benefits of vitamin E, vitamin C, and β-carotene combined antioxidants therapy in a large number of high-risk people[91].

There is increasing interest in phenolics stemming from the context of the "French paradox"[92]. This paradox refers to the correlation of a high-fat and high-cholesterol diet with a lower incidence of coronary heart disease found in Mediterranean cultures and contrasted with a higher incidence of coronary heart disease among most Western cultures. It has been shown that the French paradox may be attributable to regular consumption of red wine and that the unique antiatherogenic effects of red wine reside in the action of polyphenols. Phenolic compounds or polyphenols constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, with more

than 8000 phenolic structures currently known. Natural polyphenols can range from simple molecules (quinones, phenolic acids,) to highly polymerised compounds (lignins, melanins, tannins), with flavonoids such as flavonols, flavones, isoflavones, flavonones, flavanols and anthocyanins representing the most common and widely distributed sub-group [93] **(Table 2)**. Phenolics are therefore an integral part of the diet, with significant amounts being reported in vegetables, fruits, teas and traditional plants[94-97]. Although the dietary intake of phenolics varies considerably among geographic regions, it is estimated that daily intake range from about 20 mg to 1 g, which is higher than that for Vitamin E[98]. Epidemiological evidence indicates that consumption of fruit, vegetables and teas may reduce the risk of cardiovascular disease and it is increasingly suggested that this may due to their antioxidants that include ß-carotene, vitamin C, vitamin E and polyphenolics. Dietary antioxidant phenolics may quench reactive oxygen and nitrogen species and, hence potentially modify pathogenic mechanisms relevant to cardiovascular disease. The effectiveness of a dietary antioxidant will depend on a number of factors, such as which ROS or RNS is being scavenged, how and where they are being generated and the accessibility of the antioxidant to possible sites of damage[99]. Many phenolic compounds have been shown to have antioxidant activity in vitro [100] and several observational studies support their role in potentially protecting against cardiovascular disease[101,102]. However, not all epidemiological studies have found a protective effect of dietary phenolics against heart disease[102, 103]. In vitro studies have used various systems to oxidize LDL and then measure prevention of oxidation after inclusion of phenolic compounds like cinnamic acids [104]. A range of other compounds such as ellagic acid, dimethydiisoeugenol, phenolic rich extracts derived from olive oil, wine, grape, apple and blackcurrent juices have been shown to act as potent antioxidants inhibiting LDL oxidation *Ex vivo* and/or increasing plasma antioxidant capacity[99] .Tea polyphenols have the inherent capacity to inhibit the development of atherosclerotic lesions by down-regulating genes controlling lipid metabolism, cytokine production and cellular activity within the arterial wall namely genes coding for PPAR-γ, CD36, LXR-α, C-myc coupled with the up-regulation of genes coding for LDL-R and PPAR-α at the transcriptional level[105]. Grape seed proanthocyanidin extract (GSPE), a mixture of 75-80% oligomeric proanthocyanidin and 3-5% monomeric proanthocyanidin has proved its efficacy against the incidence of ischemia-reperfusion injury, apoptosis of cardiomyocytes and reduction of foam cell development. The mechanistic pathways of cardioprotection exerted by the proanthocyanidin rich extract included (1) potent hydroxyl and other free radical scavenging abilities; (2) anti-apoptotic,anti-necrotic and anti-endonucleolytic potentials; (3) modulatory effect on apoptotic regulatory bcl-X_L, p53 and c-myc genes; (4) cytochrome P450 2E1 inhibitory activity; (5) inhibitory effects on proapoptotic, cardioregulatory genes c-JUN and JNK-1 and (6) inhibition of vasoconstriction of vascular smooth muscle and endothelium[106,107].

CONCLUSION:

The implication of oxidative stress in the etiology of several chronic and acute degenerative disorders suggests that antioxidant therapy represents a promising avenue for treatment. Strategies for the intervention and prevention of cardiovascular disease require an understanding of the basic molecular mechanism (s) by prophylactic agents (synthetic antioxidants, dietary antioxidant factors from food plants and medicinal plants) that may potentially prevent or reverse the promotion or progression of the disease. It remains unequivocal that emerging scientific support for health claims and identification of active functional ingredients needs to be balanced by addressing toxicological concerns[108]. The real proof of efficacy for existing or novel compounds/extracts should emanate from a demonstration of clinical efficacy on defined therapeutic categories[109]. In this respect, the outcome of one such trial conducted on the Mauritian population on the effects of Mauritian black tea on markers of oxidative stress leading to cardiovascular disease is currently much awaited.

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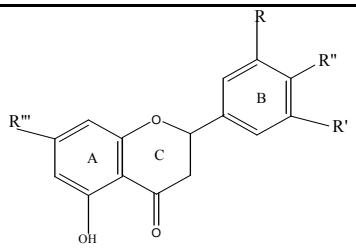
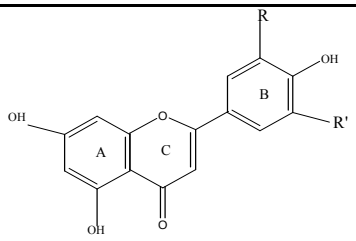
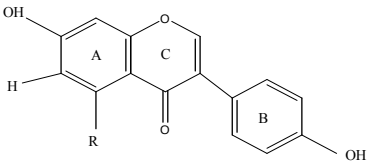
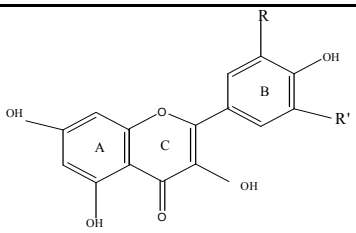
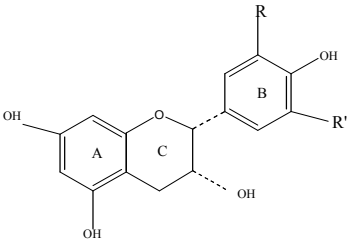
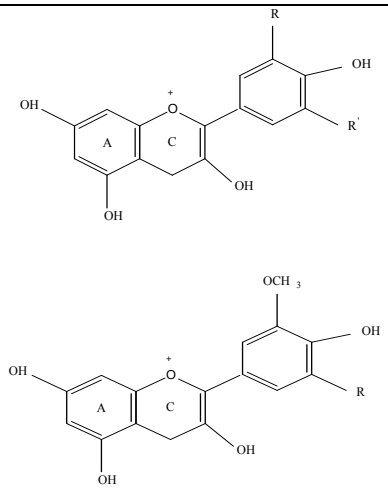
Table 2: Chemical structures of flavonoids and some examples

Classes	Structural Formula	Examples
Flavanones		R=R^1=H, R^{11}=R^{111}= OH; Naringenin R= OH, R^1=H, R^{11}=R^{111}= OH; Eriodyctiol R=R^1=OH, R^{11}=R^{111}= OH; 5^1-OH- Eriodyctiol
Flavones		R=R^1=H; Apigenin R= OH, R^1= H; Luteolin R=R^1=OCH_3; Tricetin
Isoflavones		R = H; Daidzein R = OH; Genistein
Flavonols		R=R^1=H; Kaempferol R= OH, R^1= H; Quercetin R=R^1=OH; Myricetin
Flavanols		R= OH, R^1= H; (+)-Epicatechin R=R^1=OH; (+)-Epigallocatechin R=OH, R^1=H; (–)-Catechin R=R^1=OH; (–)-Gallocatechin

Anthocyanidins		R= OH, R^1= H; Cyanidin R= R^1= OH; Delphinidin R= R^1 = OCH_3; Malvidin R= R^1= H; Pelargonidin R= H; Peonidin R= OH; Petunidin

LIST OF ABBREVIATIONS:

Ang II: Angiotensin II; AP-1: activator protein 1; AT1R: Angiotensin II type 1 Receptor; ECM: extracellular matrix; ERK: extracellular receptor kinase; ET-1: Endothelin-1; HOCl: Hypochlorous acid; HMG-CoA: 3-hydroxy 3-methylglutaryl coenzyme A; ICAM: intercellular adhesion molecule; IGF-1: insulin-like growth factor; IL-1: Interleukin-1; JNK: Jun N-terminal kinase; LDL: low density lipoprotein; MAPK: Mitogen-activated protein kinase; MCP-1: monocyte chemoattractant protein-1; MMPs: matrix metalloproteinases; NF-κB: nuclear factor κB; oxLDL: oxidized low-density lipoprotein; PDGF: platelet-derived growth factor; PECAM: platelet endothelial cell adhesion molecule; PPAR-γ: peroxisome proliferator-activated receptor-γ; Ras: small G-protein; RNS: reactive nitrogen species; ROS: reactive oxygen species; SOD: superoxide dismutase; TNFα: tumor necrosis factor alpha; VCAM: vascular cell adhesion molecule; VEGF: vascular endothelial growth factor; VSMC: vascular smooth muscle cell.

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