



Journal of
**Pharmacology and
Toxicology**

ISSN 1816-496X



Academic
Journals Inc.

www.academicjournals.com

Review Article

Endothelial Dysfunction, Oxidative Stress and Inflammation: Implications in Atherogenesis, Cardiovascular Diseases and Gene Targeted Therapeutic Approach

¹Akeem O. Lawal, ¹Dare M. Oluyede, ¹Lateefat T. Olumegbon, ¹Monsurat O. Adebimpe, ²Olamide O. Crown and ³Emmanuel Salako

¹Bioinformatics and Molecular Biology Unit, Department of Biochemistry, Federal University of Technology Akure, P.M.B. 704, Akure, Ondo, Nigeria

²Department of Chemistry, Physics and Atmospheric Sciences, Jackson States University, Jackson, Mississippi, United States

³Walden University, Minneapolis, Minnesota, United States

Abstract

Studies have shown that the transition of endothelium from the quiescent to the activated state plays a significant role in the aetiology of cardiovascular disease and atherosclerosis. The presence of excess reactive oxygen species (ROS), either due to the presence of pro-oxidant agents or in the presence of risk factors such as diabetes, hyperlipidemia, smoke etc., has been implicated in endothelium activation with the consequent dysfunctioning of the endothelium. It has been shown that the nitric oxide (NO) synthesized by the endothelium nitric oxide synthase (eNOS) plays important role in maintaining endothelium integrity by modulating the vascular tone. The presence of excessive ROS can interact with the NO with consequent production of peroxynitrite to produce other ROS and pro-inflammatory cytokines in cascade reactions. These, together with other risk factors have been implicated in ED-mediated cardiovascular disease. In this review, the data supporting the role of oxidative stress and inflammation in ED-induced cardiovascular disease and atherosclerosis was examined. Therapeutic approaches using genetics, phytochemicals and pharmaceuticals methods, in regulating the effects of ROS in ED-mediated cardiovascular morbidity and mortality and atherosclerosis were also examined.

Key words: Cardiovascular diseases, atherosclerosis, oxidative stress, inflammatory cytokines, antioxidants, endothelial dysfunction, circulatory system

Citation: Lawal, A.O., D.M. Oluyede, L.T. Olumegbon, M.O. Adebimpe, O.O. Crown and E. Salako, 2023. Endothelial dysfunction, oxidative stress and inflammation: Implications in atherogenesis, cardiovascular diseases and gene targeted therapeutic approach. J. Pharmacol. Toxicol., 18: 42-52.

Corresponding Author: Akeem O. Lawal, Bioinformatics and Molecular Biology Unit, Department of Biochemistry, Federal University of Technology Akure, P.M.B. 704, Akure, Ondo, Nigeria Tel: +2348107773102

Copyright: © 2023 Akeem O. Lawal *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The endothelium forms the biological and physical barrier between the blood in the lumen of the circulating blood vessel and the rest of the blood vessel wall. It serves as the first line of defense in the vascular wall against toxic chemicals in the circulatory blood¹. Maintaining endothelial integrity is therefore paramount to the function and integrity of the circulatory system and vascular wall integrity and function. Endothelial Dysfunction (ED) is a reflection of the balance between the repair and injury to the endothelial layer². An excessive injury that overwhelmed repair can lead to ED. Studies have shown that excessive reactive oxygen species (ROS) and inflammation cytokines production activated by the presence of chemicals and xenobiotics drive endothelial from the quiescent state toward the one that involves host defense response, which is the hallmark of ED in a chronic state^{3,4}. Hence, ED plays a significant role in the aetiology of cardiovascular diseases and atherosclerosis- a chronic inflammatory disease characterized by the deposit of lipids and fibrous elements in the large arteries. Consequently, many studies have highlighted the generation of reactive oxygen species and the production of pro-inflammatory cytokines in endothelial dysfunction^{5,6}. This paper will focus on data supporting the association between oxidative stress, inflammation and endothelial dysfunction in cardiovascular diseases and atherosclerosis. In addition, data supporting the application of antioxidants gene modulation as an effective therapy in preventing and ameliorating the effects of ED were also examined.

Endothelial biochemistry and cardiovascular health

Endothelial dysfunction: Endothelial Dysfunction (ED) is an important indicator of cardiovascular disease, predicts its prognosis and it's closely associated with the development of arteriosclerosis⁷). The term ED could be described as a "loss of function" of any of the numerous activities of the endothelium. In the context of vascular diseases, however, endothelial dysfunction describes reduced dilatory capacities, particularly reduced nitric oxide (NO) activity^{2,8,9}.

Vascular Endothelial functional alteration is the earliest stage in endothelial dysfunction and is involved with all forms of cardiovascular disease^{2,10}, especially in the presence of risk factors such as obesity, resistance to insulin and diabetes type 2¹¹. It has been reported that several factors that contribute directly to ED are major biomarkers that are implicated in the risk factors mentioned earlier¹². These biomarkers include but are not limited to hyperglycemia, low

High-Density Lipoprotein (HDL) cholesterol, high triglycerides, high blood pressure, high homocysteine levels, low vitamin D and high lipoprotein-associated phospholipase A2, elevated oxidized LDL and LDL¹³. Furthermore, the levels of these biomarkers are modulated by impairment of NO bioavailability, in addition to reduction in endothelium-mediated vasorelaxation, hemodynamic deregulation, impaired fibrinolytic ability, growth factor overproduction, oxidative stress, inflammatory and adhesion molecules genes induction^{7,14} and vice versa. Regulation of NO could be regarded as one of the key or central regulatory mechanisms that links or connects cardiovascular disease and its predisposing factors through endothelial dysfunction.

Endothelial dysfunction biomarkers: Adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) and E-selectin, among others, are typical plasma biomarkers of ED. The expression of these molecules at the surface of endothelial cells (ECs) increased in response to stimuli and inflammatory cytokines production with a consequent increase in leukocyte binding to the surface of the ECs. Studies have shown an increase in E-selectin and ICAM-1 level to be associated with an increased tendency of coronary heart disease^{15,16}. Liu *et al.*¹⁷, have shown that an increase in asymmetric dimethylarginine (ADMA) protein negatively correlates with NO levels and thus, could serve as a plasma biomarker of ED, as increased levels positively correlated with cardiovascular risk factors such as diabetes mellitus, hypertension, chronic kidney disease and dyslipidemia^{17,18}. Apart from these, other indicators of endothelial damage and repair, including inflammatory cytokines such as IL-1, IL-8, IL-6 and TNF α , could serve as biomarkers of ED¹⁶.

Loss of ECs is a hallmark of ED and atherogenesis^{19,20}. This loss is assessed by the degree of EC apoptosis. When stimulated by activating agents or during apoptosis, submicroscopic membranous vesicles are shed directly from the ECs and these endothelial microparticles carry proteins and phospholipids of the parent cells such as CD31^{21,22}, which differentiate EC-derived microparticles from microparticles derived from erythrocytes, leukocytes or platelets²³. Indeed, the continuous damage of the vascular endothelium is a key factor, in atherosclerosis initiation and progression, leading to ED. The elevated level of circulating endothelial microparticles is found in patients with cardiovascular risk factors such as diabetes, hypertriglyceridemia and acute coronary^{11,24}. Thus, circulating microparticles carrying CD31+ may serve as a novel marker of vascular ED.

Endothelial, vascular tone and vascular homeostasis: The endothelium once referred to as a 'nucleated cellophane wrapper' of the vascular tree, is a monolayer of cells covering the vascular lumen, which was once thought to be relatively inactive and considered as a mere physical barrier between circulating blood and the underlying tissues. It was not until later, that endothelial cells were discovered to be metabolically active with important paracrine, endocrine and autocrine functions, needed for the maintenance of vascular homeostasis under physiological conditions²⁵.

Endothelial cells (ECs) perform many physiological functions (Fig. 1)²⁶. These functions include, maintenance of vascular tone homeostasis (producing a balanced amount of vasodilators and vasoconstrictors), regulation of antithrombotic, procoagulant and growth factors production and production of inflammatory mediators^{2,10}. In addition, ECs help in cell adhesion, growth and remodelling of vascular cells, maintenance of vascular integrity, immune responses, angiogenesis, vascular permeability and homeostasis²⁷. The endothelium plays a pivotal role in the regulation of vascular tone (as shown in Fig. 1) by producing several vasodilators such as prostacyclin and NO and vasoconstrictors such as angiotensin-converting enzymes and endothelin, which regulate thrombosis, vasomotor tone and maintenance of blood fluidity^{28,29}. The NO is a vasodilator, synthesized from L-arginine, by the action of endothelium NO synthase (eNOS), in the endothelial cells. The synthesized NO is then diffused into the Vascular Smooth Muscle Cells (VSMC) where, it activates cyclic guanosine monophosphate (cGMP) with the consequent dilation of the VSMC^{30,31} (Fig. 2). In addition, ECs are also involved in smooth muscle cells proliferation and migration via balanced vasoconstriction and vasodilation as well as platelets aggregation and adhesion^{2,32} and thrombogenesis. Thus, unsettling this tightly regulated equilibrium leads to endothelial dysfunction.

Endothelial dysfunction, oxidative stress and inflammation:

Endothelium, being an emergent and complex system, is multifunctional, highly distributed in space and has an enormous behavioural repertoire, which makes it a powerful organizing system in human health and disease. It is also involved in numerous pathological states either as primary determinants of physiopathology or as victims of collateral damages¹¹. Endothelial dysfunction is best described as the diminished production or availability of nitric oxide (NO) and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors, such as endothelin-1 (ET-1), angiotensin and oxidants². The NO, generated by the conversion of the amino acid L-arginine to

NO and L-citrulline by the enzyme NO synthase (NOS), is the key endothelium-derived relaxing factor that plays a pivotal role in the regulation of vascular tone and vasomotor function²⁹. Little amounts of NO produced by endothelial cells give rise to smooth muscle relaxation and vasodilation and are anti-thrombogenic to platelets (Fig. 2). Thus, the onset of myocardial ischemia occurs when there is impairment in endothelium-dependent vasodilation in the coronary arteries.

Endothelial dysfunction and oxidative stress: Oxidative stress is a unifying mechanism involved in the injury of many types of disease processes. It describes the condition of an imbalance between the pro-oxidants (generation of ROS) and the antioxidant defence systems in the body so that the latter becomes overwhelmed³³. The ROS are a family of highly reactive species that are formed either enzymatically or non-enzymatically in mammalian cells and cause cell damage either directly or through behaving as intermediates in diverse signaling pathways³⁴. The generation of water from oxygen in mitochondria is an enzymatic process in which intermediates of oxygen reduction do not leave the system before the process is finished. The ROS played a pivotal role in the pathogenesis of endothelial dysfunction by causing progressive deterioration in the endothelial cells (ECs) through dysfunction, apoptosis and pro-inflammation of the ECs^{8,35}. In the presence of cardiovascular risk factors, such as diabetes, smoking, ageing and prooxidants such as air particulate matter, heavy metals and polycyclic hydrocarbons (PAHs), the endothelium can generate excess ROS and proinflammatory cytokines leading to vasomotor dysfunction and fibrinolytic imbalance with consequent myocardial ischemia, infarction and arrhythmia (Fig. 3)^{36,37}. We have shown that exposure of apolipoprotein E deficient (ApoE^{-/-}) mice to diesel exhaust particles by oropharyngeal aspiration results in an increase and destabilization of atherosclerotic lesions with other cardiovascular effects, which is accompanied by increased expressions of antioxidant genes such as HO-1, NQO1 and Nrf2, probably in response to increased oxidative stress³⁸.

The function of the endothelium is pivotal to cardiovascular integrity, hence endothelial dysfunction is an important factor in cardiovascular disease aetiology and atherogenesis. It is well-established that oxidative stress plays a central role in endothelial dysfunction and thus, in the development and pathogenesis of cardiovascular disease and atherosclerosis⁸. Endothelial function impairment involves several mechanisms, of which NO production impairment or ROS (main superoxide) production escalation plays a

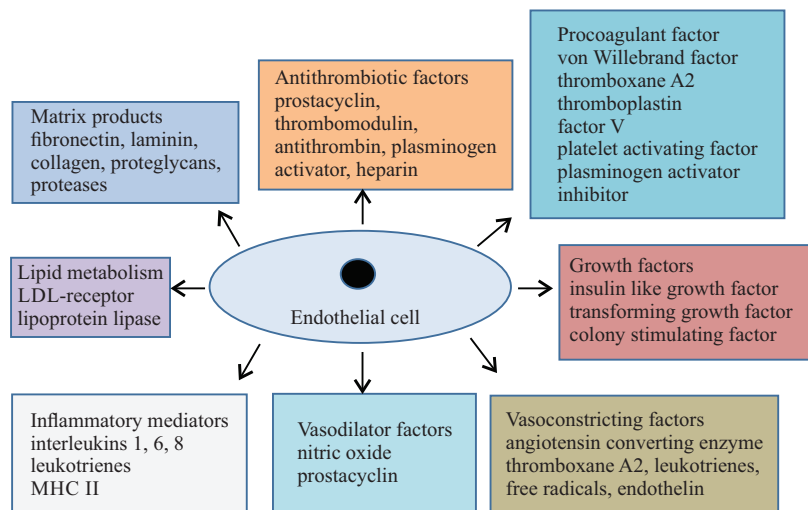


Fig. 1: Metabolic and synthetic functions of endothelial cells

ECs secrete a large variety of mediators that can influence cellular functions throughout the body and adapted from Triggle *et al.*²⁷

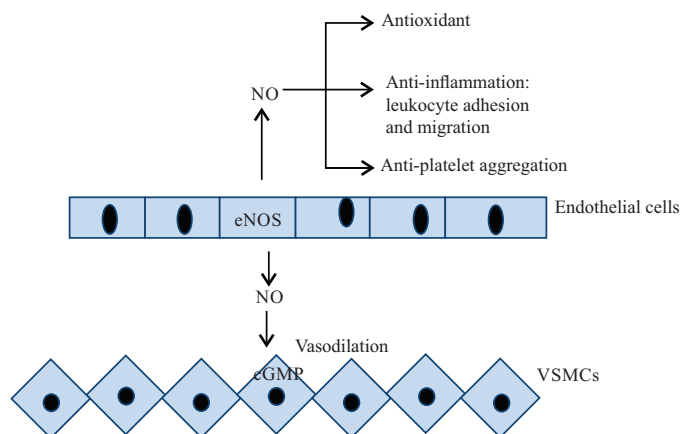


Fig. 2: NO production in endothelial cells

NO synthesized in the endothelial cells activates cGMP in the VSMCs resulting in the vasodilation of the latter and modified from Ambrosino *et al.*³²

prominent role³⁹. Indeed, the inactivation of NO by increasing oxidative stress is one important mechanism by which the endothelium-dependent vasodilation process is impaired. Cardiovascular risk factors, such as hypertension, diabetes, ageing, smoking and other inflammatory biomarkers, which may cause NO and ROS production dysregulation may be proathrogenic⁴⁰.

Under physiological conditions, endothelial cells produce reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in a careful and tightly regulated manner for use as second messengers in redox signaling pathways. However, in vascular disease (s), the ROS in vascular cells is relatively overproduced such that these molecules overpower the cellular antioxidant pathways. The NO helps in maintaining the integrity of the vascular wall. However, in the

presence of ROS such as peroxynitrite ($ONOO^-$) and decrease arginine, superoxide anions ($O_2^{\cdot-}$) production increases due to the uncoupling of eNOS. Hence, more peroxynitrites are produced as a result of excessive $O_2^{\cdot-}$ reaction with NO, thereby causing structural and functional alterations of ECs (Fig. 4)⁴¹. In addition, the iron-catalyzed Haber-Weiss reaction between O_2 and H_2O_2 , which gives rise to hydroxyl radicals ($OH^{\cdot}$) also contributes to ECs dysfunction (Fig. 4)⁴². Peroxynitrite and $OH^{\cdot}$ are extremely powerful oxidizing species and, along with $O_2^{\cdot-}$ and H_2O_2 , cause endothelial dysfunction through direct oxidative damage to cellular macromolecules, impairment of the NO signaling pathway and activation of pro-inflammatory signaling cascades. Research findings suggested that the elevated ROS production in vascular pathophysiology is the result of a

Cardiovascular risk factors (such as diabetes, smoking, ageing),
Pro-oxidants (such as air particulate matter, heavy metals, PAHs)

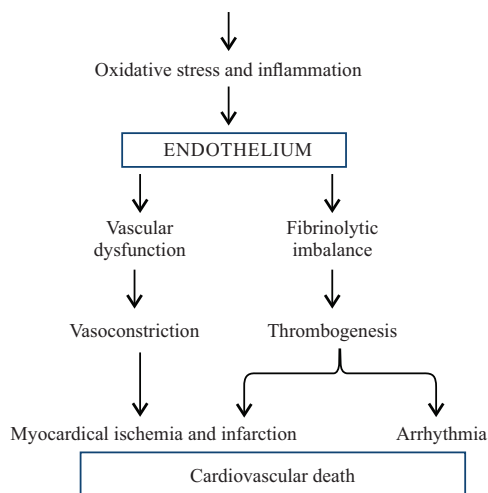


Fig. 3: Oxidative stress-induced endothelium dysfunction mediates cardiovascular events

Presence of prooxidants activates the endothelium due to oxidative stress and proinflammatory cytokines production with the consequent cardiovascular events such as myocardial ischemia and infarction and modified from Mills *et al.*³⁷

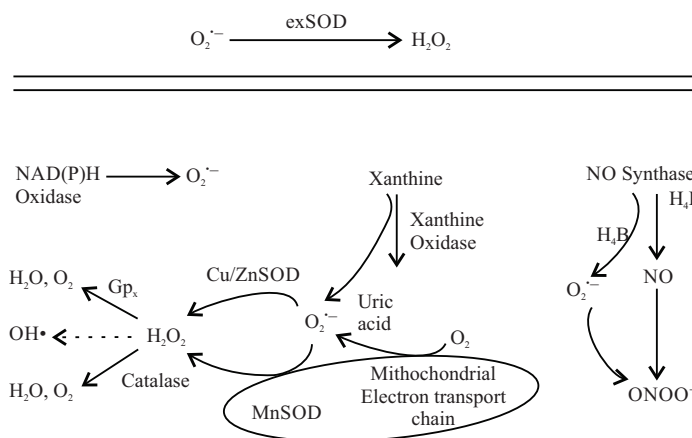


Fig. 4: Reactive oxygen species (ROS) production in vascular cells

Presence of a varied number of oxidant and antioxidant enzymes in vascular tissues contributes significantly to ROS production and elimination, SOD, superoxide dismutase, exSOD, extracellular SOD, $\text{O}_2^{\cdot -}$, Superoxide, $\text{OH}^{\cdot}$, hydroxyl radical, $\text{ONOO}^{\cdot -}$, peroxynitrite, H_2O_2 , hydrogen peroxide, H_2B , tetrahydrobiopterin, MnSOD, manganese SOD, Cu/ZnSOD, copper/zinc SOD and adapted from Wassmann *et al.*⁴¹

complex feed-forward mechanism whereby a primary source of ROS (NADPH oxidases) leads to dysfunction of endothelial nitric oxide synthase, xanthine oxidase and the mitochondrial electron transport chain, so that these enzymes become secondary sources of ROS and major contributors to vascular oxidative stress (Fig. 4)⁴³.

Endothelial dysfunction and inflammatory stress: The induction of atherosclerotic indices (such as plaques and lesions formation) in the vascular system is mediated by increased production of inflammatory cytokines irrespective of the risk factor³¹. Inflammation is an adaptive response of the

cells to deal with inflammatory and oxidative agents and debris in the vascular system and is an important step toward blood vessel repair and maintenance of tissue integrity. The increased inflammatory cytokines production due to the persistent presence of atherosclerotic protagonists in the vascular system results in the disruption in the normal function of the endothelial cells.

The presence of neutrophils and fluid protein exudates can be used to determine an acute inflammation. Inflammation is terminated when the injurious stimulus is removed and all the mediators are dissipated or inhibited. The three stages involved in acute inflammation in a vessel are

vasodilation, increased permeability of microvasculature and vascular stasis⁴⁴. The delayed sustained response is observed as endothelial cells undergo cytoskeletal changes that disrupt junctions in venules and capillaries². The factors involved in the delayed sustained response are interleukin (IL)-1, Tumour Necrosis Factor-Alpha (TNF- α), interferon-gamma (INF- γ), hypoxia and sublethal injury. Cytokines are produced mainly by lymphocytes and macrophages after stimulation by injury, toxins or inflammatory mediators and they include lymphokines, monokines, chemokines, colony-stimulating factors, interleukins and growth factors.

The production of cytokines, chemokines and adhesion molecules in an activated endothelium contributes to cardiovascular morbidity and atherogenesis⁸. Furthermore, oxidative stress via increased ROS production could trigger excessive production of inflammatory cytokines such as IL-leading to ED due to the over-production of MCP-1, IL-6 and VCAM-1³¹.

Endothelial dysfunction and therapeutic approaches:

Different enzymes expressed in vascular cells play a significant role in the production and removal of ROS. Thus, alteration of the activity and expression of these enzymes (i.e oxidants and antioxidants) may cause an imbalance in the redox status, which may cause oxidative stress in the vascular cells (Fig. 4). Antioxidants, generally, can be grouped into endogenous and exogenous depending on whether they are produced within the body or obtained from an external source. The body relies on several endogenous, as well as dietary antioxidants, defence mechanisms to help protect against free radical-induced cell damage. Glutathione peroxidase, catalase and superoxide dismutase (SOD) are antioxidant enzymes that metabolize oxidative toxic intermediates and as such require micronutrient cofactors such as selenium, iron, copper, zinc and manganese for optimum catalytic activity. A study suggested that an inadequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defence mechanism⁴⁵. The exogenous antioxidants are sources or provided for through food or supplements as they cannot be produced by the body. These supplements include vitamins C and E, carotenoids, trace metals (selenium, manganese and zinc), flavonoids, omega-3 and omega-6-fatty acids etc. Vitamin C also known as ascorbic acid is a water-soluble vitamin, which is a labile molecule that may be lost during cooking⁴⁶.

Some studies have found the consumption of flavonoid-rich fruit and vegetables, potassium and arginine supplementation help to restore impaired endothelial function⁴⁷. So further understanding of its mechanisms of

action and possible therapeutic targets will be of great importance. Reduction in reactive oxygen species (ROS) levels by vitamins A, C and E help to improve endothelial function with a consequent reduction in cardiovascular events.

Furthermore, data have revealed the relationship between increased oxidative stress, hypercholesterolemia and endothelial dysfunction². Supposed mechanisms for endothelial dysfunction include poor utilization of L-arginine substrate, impaired signal transduction of membrane receptors that enhance endothelial NO synthesis and more ADMA concentrations that inhibit NO synthase and later reduce NO production. In addition, oxidative stress also leads to a loss in NO level due to oxidative modification³¹.

The precise mechanisms by which antioxidants modulate endothelial function are unclear. Antioxidants may be protective against oxidative stress and preserve NO by scavenging ROS and inhibiting oxLDL formation. Vitamin E alone reduces ROS and apoptosis in endothelial cells that are induced by oxLDL⁴⁸. Furthermore, the combination of vitamin C and E has been shown to protect endothelial cells from the cytotoxic effects of oxLDL⁴⁹. Antioxidants may also enhance endothelial NO synthase (eNOS) activity. It has been shown that eNOS activity is impaired in hypercholesterolemia⁵⁰. Loss of eNOS in the endothelium coupled with impaired NO synthesis in hypercholesterolemic vessels may cause endothelial dysfunction⁵¹.

Effects of pharmaceutical and phytochemical sources of antioxidants on ED:

Several pharmaceutical and phytochemical molecules have been reported to protect against free radical attack and oxidative stress in endothelial cells^{4,52}. Plants, as an indispensable constituent of the human diet supplying the body with vitamins, mineral salts and certain hormone precursors as well as protein and energy, contain hundreds of phytochemicals such as flavonoids and phenolic acids as well as their derivatives, which have previously been shown to have high antioxidant activity⁴⁹. Phytochemicals are bioactive compounds found in vegetables, fruits, cereals grains and plant-based beverages such as tea and wine. Studies have shown that the consumption of these bioactive compounds is linked with a reduction in the risk of several types of chronic diseases due (in part) to their antioxidant and free radical scavenging effects^{47,49}. Recent research has also highlighted their potential role in improved endothelial function and increased vascular blood flow⁴⁹. For example, resveratrol, a polyphenolic compound found in grapes and wine, is a scavenger of hydroxyl, superoxide and metal-induced radicals⁵³. It, therefore, contributes to red

wine's potential to prevent human cardiovascular disease. Resveratrol protects against cardiac ischemia/reperfusion injury⁵⁴ and also activates adenosine receptors, stimulates nitric oxide (NO) release and induced antioxidative enzymes^{55,56}. Resveratrol has been shown to upregulate catalase and HO-1 in rat aortic segments and vascular tissues⁵⁵. In addition, treatment of cultured aortic smooth muscle and human endothelial cells with resveratrol caused an increase in NQO1, GST and catalase mRNA expressions⁵⁶.

We have shown that antioxidants from fermented and green rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* species) teas protect Human Umbilical Vein Endothelial Cells (HUVECs) from the oxidative and pro-inflammatory effects of diesel exhaust particles (DEP) by attenuating ROS generation and VCAM-1, IL-1 α , IL-6 and IL-8 genes expressions⁵⁷. In addition, these teas induced the expressions of cytoprotective enzymes such as NQO1, γ GSC and CYP1B1.

Pharmaceutical source of antioxidant has great positive effects on endothelial function. There is enough epidemiologic evidence linking the intake of pharmaceutical antioxidant vitamins such as vitamin E and vitamin C, with decreased risk of coronary artery disease^{47,49}. Consumption of vitamin E supplements causes a decrease in the risk of cardiovascular heart disease (CHD) and an inverse relationship between dietary vitamin E intake and coronary mortality has been established⁵⁸. An epidemiological study showed that dietary vitamin E intake, as opposed to supplemental vitamin E, was inversely associated with the risk of death from CHD⁵⁹. Indeed, reduced risk of all causes of mortality and coronary artery disease mortality is associated with increased vitamin E consumption (including dietary and supplements). Moreso, supplementation with both vitamin E and C further reduced the risk of CHD suggesting synergistic effects of these two vitamins.

The effects of pharmaceuticals in ED were also elucidated in our *in vitro* study. We found that the use of copper protoporphyrin (CoPPIX), a known inducer of HO-1, protects endothelial cells against the pro-inflammatory and pro-oxidant effects of DEP, leading to the survival of the endothelial cells⁴. In contrast, tin protoporphyrin, an inhibitor of HO-1, escalates the oxidative stress and proinflammatory effects of DEP on endothelial cells. Both the pharmaceutical and dietary phytochemical sources of antioxidants reduce the susceptibility of LDL oxidation and scavenge free radicals within the body, reducing the overall oxidative status of the cell. For example, vitamin E or tocopherol has been reported to inhibit LDL oxidation and leukocyte adhesion *in vitro*⁶⁰. Indeed, vitamin E is reported to reduce the inflammatory biomarkers in the serum of adults in randomized clinical trial

investigations⁶¹. Studies have shown that the antioxidants from pharmaceutical and dietary sources improve endothelium-dependent vasodilation⁴⁹. Other antioxidants aside from vitamins E and C are flavonoids, polyphenols and probucol-containing foods, such as red wine, tea, onions, garlic and apple. Furthermore, the study has reported alleviated coronary artery disease (CAD) in humans after the consumption of green tea⁶². Other fruits also have antioxidant properties as their consumption is linked with reduced (cardiovascular) CV risk⁶³. In addition, anti-oxidant vitamins are scavengers of reactive oxygen species (ROS) and thus they prevent the conversion of NO to peroxynitrite and increase NO bio availability⁸. Recently, we have also shown that naringenin, a flavanone in citrus fruit, protects the cardiovascular system from the oxidative and pro-inflammatory effects of diesel exhaust particles in exposed rats⁶⁴, thus confirming the therapeutic effect of phytochemicals in enhancing endothelium functions.

Effects of genetic modulation of antioxidant enzymes on

ED: Modulation of the antioxidant gene could proffer protection against endothelial dysfunction in an event of exposure to pro-oxidants. For example, we have previously reported that the upregulation of Heme Oxygenase -1 (HO-1) protects the endothelial cells against diesel exhaust particle (DEP)-induced oxidative stress and pro-inflammatory response⁴. A contrasting effect was seen when the HO-1 gene was silenced, with exacerbation in ROS and inflammatory cytokines production in the endothelial cells.

Effects of gene therapy directed toward modulation of key genes in ED:

Gene therapy can be said to be the therapeutic delivery of nucleic acid polymers into a patient's cells as a drug to treat diseases⁶⁵. It is also a way to correct genetic mutation arising from the source. The NOS are an important target for vascular protection² and are responsible for the production of NO as discussed earlier (Fig. 2). Endothelium-derived nitric oxide (NO) performs a plethora of vasculo-protective actions and also involves in vasorelaxation, inhibition of VSMC proliferation and migration and inhibition of platelet activation and adhesion². Thus targeting the NOS gene for therapy could be an important mechanism in the maintenance of endothelium and vascular integrity. The NOS gene transfer makes use of a mechanism to increase nitric oxide bioactivity and enhance the anti-atherogenic properties of the vessel wall. Studies have shown that delivery of the manganese superoxide dismutase gene improved vascular role in pre-arteriosclerotic carotid artery

hypercholesterolaemic in newborn⁶⁶. Another therapeutic potential is the use of autologous endothelial progenitor cells (EPC) transplantation for the repair of damaged blood vessels and bio-engineering of bio-prosthetic grafts⁶⁷. Targeted delivery of pro-angiogenic factors such as vascular endothelial growth (VEGF) may be useful in the treatment of myocardial and peripheral ischaemia. Blockage of monocyte infiltration and activation in the arterial wall by inhibition of monocyte chemo-attractant protein-1 (MCP-1) receptor activation was shown to retard the onset of atheroma and to limit the progression and destabilization of the established atherosclerotic lesion in ApoE deficient mice⁶⁸. Overexpression of antithrombotic genes of sites in the vessel wall at risk of thrombosis may be a feasible protective strategy for vulnerable plaque and prevention of acute coronary events.

CONCLUSION

Endothelial dysfunction involves the transition of endothelium from the quiescent to the activated state and plays a key role in cardiovascular disease and atherogenesis. Data have shown that the upregulation of reactive oxygen species and pro-inflammatory cytokines play important roles in Endothelial dysfunction. Thus, modulation of antioxidant gene and protein expressions can be of importance in regulating endothelium activity.

FUTURE PERSPECTIVE

Despite numerous data supporting the use of pharmaceutical and phytochemical antioxidants in protecting against endothelial dysfunction and cardiovascular disease, much work still needs to be done in the use of vector-mediated antioxidant gene delivery. This will involve understanding the detailed molecular basis in the regulation of the oxidative stress and inflammatory response in endothelium activation to proffer ways to prevent their adverse effect thereby enhancing lifespan.

SIGNIFICANCE STATEMENT

Cardiovascular diseases are the leading cause of death worldwide and an estimated 17.9 million deaths occur each year as a result of cardiovascular conditions. Endothelium cells form the first line of defense against chemical insults and free radicals attacks in the vascular system and thus play a significant role in protecting against cardiovascular morbidity and mortality. It thus becomes imperative to evaluate the

different forms of therapies that could be effective in ameliorating the adverse cardiovascular effects of toxic agents via damage to the endothelial. An in-depth insight into the different therapeutic approaches directed against the development of endothelial dysfunction shows that gene-target therapy complemented with other pharmacological and phytochemical treatments may be very effective in mitigating the development of cardiovascular conditions and thus enhancing life span and well-being.

REFERENCES

1. Ding, Y., Y. Zhou, P. Ling, X. Feng and S. Luo *et al.*, 2021. Metformin in cardiovascular diabetology: A focused review of its impact on endothelial function. *Theranostics*, 11: 9376-9396.
2. Sun, H.J., Z.Y. Wu, X.W. Nie and J.S. Bian, 2020. Role of endothelial dysfunction in cardiovascular diseases: The link between inflammation and hydrogen sulfide. *Front. Pharmacol.*, Vol. 10. 10.3389/fphar.2019.01568.
3. Akhigbe, R. and A. Ajayi, 2021. The impact of reactive oxygen species in the development of cardiometabolic disorders: A review. *Lipids Health Dis.*, Vol. 20. 10.1186/s12944-021-01435-7.
4. Lawal, A.O., M. Zhang, M. Dittmar, A. Lulla and J.A. Araujo, 2015. Heme oxygenase-1 protects endothelial cells from the toxicity of air pollutant chemicals. *Toxicol. Appl. Pharmacol.*, 284: 281-291.
5. Incalza, M.A., R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola and F. Giorgino, 2018. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vasc. Pharmacol.*, 100: 1-19.
6. Checa, J. and J.M. Aran, 2020. Reactive oxygen species: Drivers of physiological and pathological processes. *J. Inflammation Res.*, 13: 1057-1073.
7. Eckers, A. and J. Haendeler, 2015. Endothelial cells in health and disease. *Antioxid. Redox Signaling*, 22: 1209-1211.
8. Lawal, A.O., L.M. Davids and J.L. Marnewick, 2016. Diesel exhaust particles and endothelial cells dysfunction: An update. *Toxicol. in vitro*, 32: 92-104.
9. Lee, G.H., T.H. Hoang, E.S. Jung, S.J. Jung and S.K. Han *et al.*, 2020. Anthocyanins attenuate endothelial dysfunction through regulation of uncoupling of nitric oxide synthase in aged rats. *Aging Cell*, Vol. 19. 10.1111/acer.13279.
10. Mangana, C., M. Lorigo and E. Cairrao, 2021. Implications of endothelial cell-mediated dysfunctions in vasomotor tone regulation. *Biologics*, 1: 231-251.
11. Kaur, R., M. Kaur and J. Singh, 2018. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: Molecular insights and therapeutic strategies. *Cardiovasc. Diabetology*, Vol. 17. 10.1186/s12933-018-0763-3.

12. Lima, B.B., M. Hammadah, J.H. Kim, I. Uphoff and A. Shah *et al.*, 2019. Association of transient endothelial dysfunction induced by mental stress with major adverse cardiovascular events in men and women with coronary artery disease. *JAMA Cardiol.*, 4: 988-996.
13. Hermans, M.P. and P. Valensi, 2018. Elevated triglycerides and low high-density lipoprotein cholesterol level as marker of very high risk in type 2 diabetes. *Curr. Opin. Endocrinol. Diabetes and Obesity*, 25: 118-129.
14. Scioli, M.G., G. Storti, F. D'Amico, R.R. Guzmán and F. Centofanti *et al.*, 2020. Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: Potential diagnostic biomarkers and therapeutic targets. *J. Clin. Med.*, Vol. 9. 10.3390/jcm9061995.
15. Mozos, I., C. Malainer, J. Horbańczuk, C. Gug, D. Stoian, C.T. Luca and A.G. Atanasov, 2017. Inflammatory markers for arterial stiffness in cardiovascular diseases. *Front. Immunol.*, Vol. 8. 10.3389/fimmu.2017.01058.
16. Steyers, C.M. and F.J. Miller, 2014. Endothelial dysfunction in chronic inflammatory diseases. *Int. J. Mol. Sci.*, 15: 11324-11349.
17. Liu, X., X. Xu, R. Shang and Y. Chen, 2018. Asymmetric dimethylarginine (ADMA) as an important risk factor for the increased cardiovascular diseases and heart failure in chronic kidney disease. *Nitric Oxide*, 78: 113-120.
18. Lorin, J., J.C. Guillard, K. Stamboul, C. Guenancia and Y. Cottin *et al.*, 2017. Increased symmetric dimethylarginine level is associated with worse hospital outcomes through altered left ventricular ejection fraction in patients with acute myocardial infarction. *PLoS ONE*, Vol. 12. 10.1371/journal.pone.0169979.
19. Chang, R., A. Mamun, A. Dominic and N.T. Le, 2021. SARS-CoV-2 mediated endothelial dysfunction: The potential role of chronic oxidative stress. *Front. Physiol.*, Vol. 11. 10.3389/fphys.2020.605908.
20. Gimbrone, M.A. and G. García-Cardeña, 2016. Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ. Res.*, 118: 620-636.
21. Fujimoto, S., Y. Fujita, T. Kadota, J. Araya and K. Kuwano, 2021. Intercellular communication by vascular endothelial cell-derived extracellular vesicles and their micrnas in respiratory diseases. *Front. Mol. Biosci.*, Vol. 7. 10.3389/fmolb.2020.619697.
22. Oggero, S., S. Austin-Williams and L.V. Norling, 2019. The contrasting role of extracellular vesicles in vascular inflammation and tissue repair. *Front. Pharmacol.*, Vol. 10. 10.3389/fphar.2019.01479.
23. Lapping-Carr, G., J. Gemel, Y. Mao and E.C. Beyer, 2020. Circulating extracellular vesicles and endothelial damage in sickle cell disease. *Front. Physiol.*, Vol. 11. 10.3389/fphys.2020.01063.
24. Miller, M., N.J. Stone, C. Ballantyne, V. Bittner and M.H. Criqui *et al.*, 2011. Triglycerides and cardiovascular disease: A scientific statement from the American heart association. *Circulation*, 123: 2292-2333.
25. Ricard, N., S. Bailly, C. Guignabert and M. Simons, 2021. The quiescent endothelium: Signalling pathways regulating organ-specific endothelial normalcy. *Nat. Rev. Cardiol.*, 18: 565-580.
26. Galley, H.F. and N.R. Webster, 2004. Physiology of the endothelium. *Br. J. Anaesth.*, 93: 105-113.
27. Triggle, C.R., S.M. Samuel, S. Ravishankar, I. Marei, G. Arunachalam and H. Ding, 2012. The endothelium: Influencing vascular smooth muscle in many ways. *Can. J. Physiol. Pharmacol.*, 90: 713-738.
28. Feletou, M. and P.M. Vanhoutte, 2006. Endothelial dysfunction: A multifaceted disorder (The Wiggers Award Lecture). *Am. J. Physiol. Heart Circulatory Physiol.*, 291: H985-H1002.
29. Vassiliou, A.G., A. Kotanidou, I. Dimopoulou and S.E. Orfanos, 2020. Endothelial damage in acute respiratory distress syndrome. *Int. J. Mol. Sci.*, Vol. 21. 10.3390/ijms21228793.
30. Fajmut, A., 2021. Molecular Mechanisms and Targets of Cyclic Guanosine Monophosphate (cGMP) in Vascular Smooth Muscles. In: *Muscle Cell and Tissue-Novel Molecular Targets and Current Advances*, Sakuma, K. (Ed.), IntechOpen, ISBN: 978-1-83968-651-1.
31. Mudau, M., A. Genis, A. Lochner and H. Strijdom, 2012. Endothelial dysfunction: The early predictor of atherosclerosis: Review article. *Cardiovasc. J. Afr.*, 23: 222-231.
32. Ambrosino, P., T. Bachetti, S.E. D'Anna, B. Galloway and A. Bianco *et al.*, 2022. Mechanisms and clinical implications of endothelial dysfunction in arterial hypertension. *J. Cardiovasc. Dev. Dis.*, Vol. 9. 10.3390/jcdd9050136.
33. Sharifi-Rad, M., N.V.A. Kumar, P. Zucca, E.M. Varoni and L. Dini *et al.*, 2020. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Front. Physiol.*, Vol. 11. 10.3389/fphys.2020.00694.
34. Sinha, N. and P.K. Dabla, 2015. Oxidative stress and antioxidants in hypertension-A current review. *Curr. Hypertens. Rev.*, 11: 132-142.
35. Zhang, W., Q. Huang, Z. Zeng, J. Wu, Y. Zhang and Z. Chen, 2017. Sirt1 inhibits oxidative stress in vascular endothelial cells. *Oxid. Med. Cell. Longevity*, Vol. 2017. 10.1155/2017/7543973.
36. Peng, Z., B. Shu, Y. Zhang and M. Wang, 2019. Endothelial response to pathophysiological stress. *Arteriosclerosis Thrombosis Vasc. Biol.*, 39: e233-e243.
37. Mills, N.L., K. Donaldson, P.W. Hadoke, N.A. Boon and W. MacNee *et al.*, 2009. Adverse cardiovascular effects of air pollution. *Nat. Clin. Pract. Cardiovasc. Med.*, 6: 36-44.

38. Miller, M.R., S.G. McLean, R. Duffin, A.O. Lawal and J.A. Araujo *et al.*, 2013. Diesel exhaust particulate increases the size and complexity of lesions in atherosclerotic mice. Part. Fibre Toxicol., Vol. 10. 10.1186/1743-8977-10-61.
39. Morris, G., B.K. Puri, L. Olive, A. Carvalho and M. Berk *et al.*, 2020. Endothelial dysfunction in neuroprogressive disorders-causes and suggested treatments. BMC Med., Vol. 18. 10.1186/s12916-020-01749-w.
40. Carnevale, R., V. Cammisotto, F. Pagano and C. Nocella, 2018. Effects of Smoking on Oxidative Stress and Vascular Function. In: Smoking Prevention and Cessation, Rajer, M. (Ed.), IntechOpen, London, UK, ISBN: 978-1-83881-574-5, pp: 25-48.
41. Wassmann, S., K. Wassmann and G. Nickenig, 2004. Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. Hypertension, 44: 381-386.
42. Manea, A., 2010. NADPH oxidase-derived reactive oxygen species: Involvement in vascular physiology and pathology. Cell Tissue Res., 342: 325-339.
43. Sena, C.M., A. Leandro, L. Azul, R. Seica and G. Perry, 2018. Vascular oxidative stress: Impact and therapeutic approaches. Front. Physiol., Vol. 9. 10.3389/fphys.2018.01668.
44. Aguilar-Cazares, D., R. Chavez-Dominguez, A. Carlos-Reyes, C. Lopez-Camarillo, O.N.H. de la Cruz and J.S. Lopez-Gonzalez, 2019. Contribution of angiogenesis to inflammation and cancer. Front. Oncol., Vol. 9. 10.3389/fonc.2019.01399.
45. Dhalaria, R., R. Verma, D. Kumar, S. Puri and A. Tapwal *et al.*, 2020. Bioactive compounds of edible fruits with their anti-aging properties: A comprehensive review to prolong human life. Antioxidants, Vol. 9. 10.3390/antiox911123.
46. Carr, A.C. and S. Rowe, 2020. Factors affecting vitamin C status and prevalence of deficiency: A global health perspective. Nutrients, Vol. 12. 10.3390/nu12071963.
47. Lidder, S. and A.J. Webb, 2013. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. Br. J. Clin. Pharmacol., 75: 677-696.
48. Boushehri, S.N., R.M. Yusof, M.N.M. Taib, K. Mirzaei, N. Yazdekhaesti and S. Akbarzadeh, 2012. Effect of vitamin supplementation on serum oxidized low-density lipoprotein levels in male subjects with cardiovascular disease risk factors. Iran. J. Basic Med. Sci., 15: 958-964.
49. Man, A.W.C., H. Li and N. Xia, 2020. Impact of lifestyles (diet and exercise) on vascular health: Oxidative stress and endothelial function. Oxid. Med. Cell. Longevity, Vol. 2020. 10.1155/2020/1496462.
50. Fuenzalida, B., B. Sobrevia, C. Cantin, L. Carvajal and R. Salsoso *et al.*, 2018. Maternal supraphysiological hypercholesterolemia associates with endothelial dysfunction of the placental microvasculature. Sci. Rep., Vol. 8. 10.1038/s41598-018-25985-6.
51. Qiang, L., J.Y. Youn and H. Cai, 2015. Mechanisms and consequences of endothelial nitric oxide synthase dysfunction in hypertension. J. Hypertens., 33: 1128-1136.
52. Tseng, C.Y., J.S. Wang, Y.J. Chang, J.F. Chang and M.W. Chao, 2015. Exposure to high-dose diesel exhaust particles induces intracellular oxidative stress and causes endothelial apoptosis in cultured *in vitro* capillary tube cells. Cardiovasc. Toxicol., 15: 345-354.
53. Gu, T., N. Wang, T. Wu, Q. Ge and L. Chen, 2021. Antioxidative stress mechanisms behind resveratrol: A multidimensional analysis. J. Food Qual., Vol. 2021. 10.1155/2021/5571733.
54. Li, T., Y. Tan, S. Ouyang, J. He and L. Liu, 2022. Resveratrol protects against myocardial ischemia-reperfusion injury via attenuating ferroptosis. Gene, Vol. 808. 10.1016/j.gene.2021.145968.
55. Gal, R., L. Deres, K. Toth, R. Halmosi and T. Habon, 2021. The effect of resveratrol on the cardiovascular system from molecular mechanisms to clinical results. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms221810152.
56. Farkhondeh, T., S.L. Folgado, A.M. Pourbagher-Shahri, M. Ashrafizadeh and S. Samarghandian, 2020. The therapeutic effect of resveratrol: Focusing on the Nrf2 signaling pathway. Biomed. Pharmacother., Vol. 127. 10.1016/j.biopha.2020.110234.
57. Lawal, A.O., L.M. Davids and J.L. Marnewick, 2019. Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* species) modulate the oxidative stress associated injury of diesel exhaust particles in human umbilical vein endothelial cells. Phytomedicine, Vol. 59. 10.1016/j.phymed.2019.152898.
58. Tobias, D.K., 2019. Vitamin E: Objective marker of healthful diet and long-term mortality. Circ. Res., 125: 41-42.
59. Shah, A.K. and N.S. Dhalla, 2021. Effectiveness of some vitamins in the prevention of cardiovascular disease: A narrative review. Front. Physiol., Vol. 12. 10.3389/fphys.2021.729255.
60. Ziegler, M., M. Wallert, S. Lorkowski and K. Peter, 2020. Cardiovascular and metabolic protection by vitamin E: A matter of treatment strategy? Antioxidants, Vol. 9. 10.3390/antiox9100935.
61. Asbaghi, O., M. Sadeghian, B. Nazarian, M. Sarreshtedari and H. Mozaffari-Khosravi *et al.*, 2020. The effect of vitamin E supplementation on selected inflammatory biomarkers in adults: A systematic review and meta-analysis of randomized clinical trials. Sci. Rep., Vol. 10. 10.1038/s41598-020-73741-6.
62. Lorenz, M., F. Rauhut, C. Hofer, S. Gwosc and E. Müller *et al.*, 2017. Tea-induced improvement of endothelial function in humans: No role for epigallocatechin gallate (EGCG). Sci. Rep., Vol. 7. 10.1038/s41598-017-02384-x.

63. Arias, A., G. Feijoo and M.T. Moreira, 2022. Exploring the potential of antioxidants from fruits and vegetables and strategies for their recovery. *Innovative Food Sci. Emerg. Technol.*, Vol. 77. 10.1016/j.ifset.2022.102974.
64. Oluyede, D.M., A.O. Lawal, M.O. Adebimpe, L.T. Olumegbon and O.O. Elekofehinti, 2021. Biochemical and molecular effects of naringenin on the cardiovascular oxidative and pro-inflammatory effects of oral exposure to diesel exhaust particles in rats. *Air Qual. Atmos. Health*, 14: 935-953.
65. Ermak, G., 2015. *Emerging Medical Technologies*. World Scientific, Pages: 152.
66. Afolayan, A.J., A. Eis, R.J. Teng, I. Bakhutashvili, S. Kaul, J.M. Davis and G.G. Konduri, 2012. Decreases in manganese superoxide dismutase expression and activity contribute to oxidative stress in persistent pulmonary hypertension of the newborn. *Am. J. Physiol.-Lung Cell. Mol. Physiol.*, 303: L870-L879.
67. Wang, W., Y. Zhang, H. Hui, W. Tong and Z. Wei *et al.*, 2021. The effect of endothelial progenitor cell transplantation on neointimal hyperplasia and reendothelialisation after balloon catheter injury in rat carotid arteries. *Stem Cell Res. Ther.*, Vol. 12. 10.1186/s13287-021-02135-w.
68. Kang, H., X. Li, K. Xiong, Z. Song and J. Tian *et al.*, 2021. The entry and egress of monocytes in atherosclerosis: A biochemical and biomechanical driven process. *Cardiovasc. Ther.*, Vol. 2021 10.1155/2021/6642927.