Current Pharmaceutical Design, **2020,** ***26*****(22):2591-2601.**
doi: 10.2174/1381612826666200318152049.

Manuscript: Recd: Nov 08, 2019. Accd: Feb 28, 2020. Publ: July 05, 2020

Mechanisms of Medicinal Plant Activity on Nitric Oxide (NO) Bioavailability as Prospective Treatments for Atherosclerosis

Khojasteh Malekmohammad[1], Robert D. E. Sewell[2] and Mahmoud Rafieian-Kopaei[3],*

[1]Department of Biology, Faculty of Basic Sciences, Shiraz University, Shiraz, Iran; [2]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, CF10 3NB. Wales, U.K; [3]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Abstract:

Background and objective**:** Atherosclerosis is one of the leading causes of human morbidity globally and reduced bioavailability of vascular nitric oxide (NO) has a critical role in the progression and development of the atherosclerotic disease. Loss of NO bioavailability, for example *via* a deficiency of the substrate (L-arginine) or cofactors for endothelial nitric oxide synthase (eNOS), invariably leads to detrimental vascular effects such as impaired endothelial function and increased smooth muscle cell proliferation, deficiency of the substrate (Larginine) or cofactors for eNOS. Many Various medicinal plants and their bioactive compounds or secondary metabolites with fewer side effects are potentially implicated in preventing cardiovascular disease by increasing NO bioavailability, thereby ameliorating endothelial dysfunction. In this review, we describe the most notable medicinal plants and their bioactive compounds that may be appropriate for enhancing NO bioavailability, and treatment of atherosclerosis.

Method**:** The material in this article was obtained from noteworthy scientific databases, including Web of Science, PubMed, Science Direct, Scopus and Google Scholar.

Results**:** Medicinal plants and their bioactive compounds influence NO production through diverse mechanisms including the activation of the nuclear factor kappa B (NF-κB) signaling pathway, activating protein kinase C (PKC)-α, stimulating protein tyrosine kinase (PTK), reducing the conversion of nitrite to NO *via* nitrate-nitrite reduction pathways, induction of eNOS, activating the phosphatidylinositol 3-kinase (PI3K)/serine threonine protein kinase B (AKT) (PI3K/AKT/eNOS/NO) pathway and decreasing oxidative stress.

Conclusion**:** Medicinal plants and/or their constituent bioactive compounds may be considered as safe therapeutic options for enhancing NO bioavailability and prospective preventative therapy for atherosclerosis.

Keywords: Nitric oxide, NO, eNOS, atherosclerosis, medicinal plants, bioactive compounds

*Address correspondence to this author at the Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran; Tel: +98 381 334 6692; Fax: +98 381 3330709; E-mail: rafieian@yahoo.com

1. INTRODUCTION

Atherosclerosis is one of the leading causes of human morbidity globally [1]. Several risk factors (smoking, hypertension, diabetes, hypercholesterolemia and age), in addition to other contributory issues (genetics, nutrition, inflammation/infection, obesity and early growth) participate in atherosclerosis by influencing nitric oxide (NO) bioavailability, levels of NO and endothelial function [2-5]. In various organisms, nitric oxide bioavailability is determined by the generation and utilization of endothelial NO [3]. Risk factors related to systemic oxidative stress and inflammation, which decrease the bioavailability of endothelial NO, lead to impaired vasodilator function [2-6]. In consequence, reduced NO bioavailability in the vessel wall is a prime characteristic of endothelial dysfunction [2,7]. NO is not only a potent vasodilator but also a multifunctional signaling molecule in the immune, nervous and cardiovascular systems [3, 8]. Originally, Endothelium-derived relaxing factor (EDRF) was discovered in the 1980s and subsequently identified as NO released from the endothelium to induce relaxation of underlying vascular smooth muscle [9-11]. NO is biologically synthesized from its amino acid precursor L-arginine by the L-arginine-nitric oxide pathway through the activity of the NO synthase (NOS) family in endothelial cells, macrophages and neurons [12, 13]. NO production occurs *via* the formation of an intermediate molecule, which is then oxidized to L-citrulline and nitric oxide. NO diffuses from the endothelium into the underlying smooth muscle cells to activate soluble guanylate cyclase (sGC). Finally, cyclic guanosine monophosphate (cGMP) formation causes vasodilatation by vessel relaxation [4]. The average degree of NO generation in the human body (70 kg) is 1.68 mmoL NO per day [14, 15].

Three distinct nitric oxide synthase (NOS) isoforms have been identified for NO generation and they are encoded by 3 genes: (1) neuronal NOS (NOS I or nNOS), which acts as a neurotransmitter in non-adrenergic and non-cholinergic neurons and producing low quantities of NO in the picomolar (pM) to nanomolar (nM) range through an intracellular calcium-dependent process. nNOS is located on chromosome 12 [5, 16, 17]. (2) Inducible NOS (NOS II or iNOS) exists in endothelial cells, smooth muscle cells, macrophages and cardiac myocytes. The gene encoding iNOS is located on chromosome 17. It produces high levels of nitric oxide in the micromolar (μM) range for a short time in a calcium-independent manner [5, 16, 18]. (3) Endothelial NOS (NOS III or eNOS) is mainly expressed in the endothelium. It is located on chromosome 7 and it also exists in the kidney, human placenta, cardiomyocytes, platelets, and some neurons. The activity of this isoform is dependent on intracellular calcium and generates NO in the pM to nM range [5, 19, 20]. The dominant NOS isoform in the vasculature is eNOS [21]. Another sub-isoform of the eNOS enzyme exists in mitochondria (mtNOS) where it generates NO [15]. Regulatory cofactors for NOS are: Ca2+/calmodulin (CaM), heat shock protein 90 (hsp90), nicotinamide-adenine-dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and tetrahydrobiopterin (BH4). These NADPH-dependent enzymes regulate at three levels, namely transcription, substrate availability, and posttranslationally [22].

The most important physiological actions of NO and its antiatherogenic properties *in vitro* stem from: Inhibiting cell-mediated LDL oxidation and Ox-LDL cytotoxicity, enhancement of vasodilatation and endothelial repair, inhibiting inflammatory cell activation, reducing thrombosis, inhibiting platelet adhesion and aggregation, reducing monocyte and leukocyte adhesion to the endothelium, inhibiting MCP-1 expression, inhibiting NF-κB activation/DNA binding through S-nitrosylation of the Cys 62 residue of the p50 subunit, inhibiting proliferation of vascular smooth muscle cells (VSMCs) through a cGMP (cyclic guanosine monophosphate) dependent mechanism, inhibiting superoxide anion production *via* a direct action on NADPH oxidase, inactivating xanthine oxidase (XO) and NADPH oxidase, preservation of the normal vessel wall structure and maintenance of metabolic and cardiovascular homeostasis [3, 4, 7, 16, 23-27]. NO induces *in vivo*

anti-atherogenic properties through modulation of endothelium-leukocyte interaction, modulation of intimal thickening, EDRF/NO and eNOS transfection [4, 28-33].

Endothelial dysfunction, a critical early event during atherogenesis, decreases the capacity of the endothelium to produce NO and its bioavailability [34]. Loss of NO bioavailability is implicated in many cardiovascular disorders such as atherosclerosis and hypertension [5, 20, 35]. The reduction of endothelial NO bioavailability is related to its reactions with reactive oxygen species (ROS), reduction in NO enzymatic synthesis [36], decrease in expression of eNOS, deficiency of substrate (L-arginine) or cofactors for eNOS, reduced capacity of endothelial cells (ECs) to synthesize and/or release NO and inactivation of generated NO by ROS [37, 38]. Furthermore, the use of NO synthesis inhibitors increases the oxidation of LDL and inhibits NO generation [39].

For these reasons, application of safe and effective strategies to raise NO activity and/or synthesis, and in order to restore normal NO homeostasis or to provide an exogenous source of NO, are regarded as beneficial in the treatment of atherosclerosis and its related diseases. Therefore, in recent years, medicinal plants and their valuable natural components have been utilized worldwide because of their therapeutic effects, low cost and fewer side effects compared to more expensive conventional drugs with adverse effects [40]. Despite widespread reports about the effects of NO on the prevention of cardiovascular diseases such as atherosclerosis along with an abundance of medicinal plants and bioactive compounds for the enhancement of NO bioavailability and treatment of atherosclerosis, there is no comprehensive literature review on this topical issue.

So consequently, this article aims to summarize effective medicinal plants and bioactive compounds that are currently available for the enhancement and improvement of NO production or availability in the human body for the treatment or prevention of atherosclerosis.

2. MATERIALS AND METHOD

The material information in this article was obtained from noteworthy scientific databases such as Web of Science, PubMed,
Science Direct, Scopus and Google Scholar. The main keywords used as searched terms were: “atherosclerosis”, “nitric oxide”, “NO bioavailability”, “reactive nitrogen species”, “medicinal plants”, and “bioactive compounds”.

3. RESULTS

3.1. Atherosclerosis and Nitric Oxide

The monolayer vascular endothelium is located between vascular smooth muscle cells and the vessel lumen [5]. Endothelial dysfunction has an important role in many cardiovascular diseases such as atherosclerosis, heart failure and hypertension [41, 42]. One of the most the important features of endothelial dysfunction is depression of the NO: sGC (nitric oxide; soluble guanylate cyclase) pathway at different levels including down-regulation of eNOS expression and activity, uncoupling of NOS, NO scavenging by oxygen-centered free radicals and reduced sensitivity of VSMCs to vasodilators [43]. Endothelial cell injury can occur by several mechanisms including oxidative stress (low density lipoprotein (LDL) oxidation and Ox-LDL formation), low shear stress within the vessel (reduction of NO synthase stimulation and NO generation) [5, 34], production of endothelium-derived constricting factors and impaired endothelial cell signal transduction [23].

In the first mechanism, a disequilibrium between excessive generation of ROS and/or RNS [*e.g.* hydroxyl ions (OH-), superoxide anions (O2. −), hydrogen peroxide (H2O2)] and the antioxidant defense system [*e.g.* glutathione peroxidases (GPX), superoxide dismutases (SOD)] induces oxidative stress [44]. The sources of ROS and RNS production such as cyclooxygenase derived prostaglandins, lipoxygenase, myeloperoxidase (MPO), NAD(P)H oxidase and xanthine oxidase

(XO) contribute to atherosclerosis, reduced NO availability, uncoupling of eNOS and endothelial dysfunction [36].

Moreover, rapid interaction and combination of NO with O2 - (superoxide) forms peroxynitrite (ONOO-) and then initiates lipid peroxidation [34]. In essence, oxidative stress leads to the formation of reactive nitrogen/oxygen species, induction of protein nitration, reduction of NO bioavailability and a decrease in NO levels through eNOS uncoupling [3, 35, 45-47]. eNOS uncoupling induces the production of superoxide instead of NO by NOS enzymes in the absence of substrate, L-arginine, or the eNOS cofactor tetrahydrobiopterin (BH4) [48-50]. In this condition, oxidative stress and LDL oxidation increase within endothelial walls. Then, Ox-LDL damages endothelial cells by impairing eNOS bioactivity [51]. Ox-LDLs accumulate in the endothelial cells, intima, and smooth muscle cells (SMCs). Ox-LDL and cytokines induce the expression of VCAM-1 and ICAM-1 on endothelial cells (ECs) as a result of a reaction of ECs and SMCs by secreting monocyte MCP-1 and production of cytokines and growth factors. Macrophages migrate into the EC, intima and SMC layers and Ox-LDL uptake occurs, leading to the development of fatty streaks and foam cells [52]. Macrophages and foam cells contribute to the production of ROS, which can oxidize BH4 into dihydrobiopterin (BH2). BH4 is a prominent cofactor for eNOS function and maintaining eNOS dimerization. This cofactor transfers electrons from eNOS reductase to the oxygenase domain, which has a binding site for L-arginine and BH4. Electron transference converts the substrate, L-arginine, to NO and L-citrulline. Thus, the generation of NO leads to SMC relaxation, vasodilation, control of vascular tone plus platelet function and atheroprotection through soluble guanylate cyclase (sGC) and guanosine-3',5-monophosphate (cGMP) signaling. In contrast, BH4 oxidation to dihydrobiopterin (BH2) and biopterin by peroxynitrite and other reactive oxygen species along with a reduction of BH4 bioavailability cause eNOS to be unstable and uncoupled. So BH2 is competitively replaced with eNOS-bound BH4 and this form of eNOS synthesizes superoxide instead of NO [53]. Also, L-arginine is converted to superoxide instead, and its conversion subsequently causes LDL oxidation and endothelial dysfunction [35, 54-56]. Finally, atherosclerotic plaque formation and thrombosis occur in the late stage of atherosclerosis [52].

Shear stress is a key activator of eNOS in normal physiological conditions [44] and it participates in the modulation of eNOS expression. Shear stress can induce both eNOS transcription and stabilization of eNOS mRNA. Arterial shear stress (> 15 dyne/cm2 or long-term laminar shear stress in endothelial cells) can increase eNOS expression, NO availability, and produce atheroprotective effects. Alternatively, low shear stress (< 4 dyne/cm2) or short term shear stress decreases eNOS expression and increases NAD(P)H oxidase-dependent superoxide production in human aortic endothelial cells so on the whole, it has proatherosclerotic properties [36, 57, 59]. The action mechanisms of shear stress in releasing NO involve ion channel activation (opening chloride, potassium and calcium ion channels) and activating signaling pathways such as phosphorylation of eNOS protein or serine/threonine protein kinase B (Akt) and enhanced expression of eNOS mRNA and protein to maintain long term production of nitric oxide [5].

3.2. NO Signaling Pathways

Different NO signaling pathways including eNOS/NO, iNOS/NO and nNOS/NO can impact NO synthesis to ameliorate atherosclerosis. The eNOS/NO signaling pathway consists of the phosphatidylinositol 3-kinase (PI3K)/serine threonine protein kinase B(AKT)/eNOS pathway, the Ca(2+)/ calmodulin-dependent kinase II (CaMKII) or calmodulin-dependent protein kinase ($Ca(2^+)$/CaMKII or CaMKK/AMPK/AKT/eNOS) pathway, nuclear factor erythroid 2-related factor 2/ Heme oxygenase-1/ endothelial nitric oxide synthase (Nrf2/HO-1/eNOS) pathway and signal transducer and activator of transcription 3/Dimethyl Arginine Dimethyl Amino Hydrolase/ Asymmetric Dimethyl Arginine/ eNOS pathway (STAT3/DDAH/ADMA/eNOS pathway). The iNOS/NO signaling pathway consists of the nuclear factor kappa B (NF-κB)/iNOS pathway, the IκB kinase complex/Inhibitory subunit of NF-Kb/ NF-κB/iNOS pathway (IKK/IκBα/NF-κB/iNOS pathway), the janus Kinase 2/ Signal Transducer and Activator of Transcription 3/iNOS pathway (JAK2/STAT3-iNOS pathway), mitogen-activated protein kinase, extracellular regulated protein1/2,

c-JunNterminalkinase 1/2 and the p38 (MAPK, ERK1/2, JNK1/2 and p38) iNOS pathway and the nuclear factor erythroid 2-related factor 2/Heme oxygenase-1/ Inducible nitric oxide synthase (Nrf2/HO-1/iNOS) pathway. Extracellular regulated protein (ERK)1/2/nNOS is the main nNOS/NO signaling pathway (Fig. **1**) [1].

3.3. NO Inhibitors or NO Reducers

Many elements including the following factors are implicated in the reduction of NO production, eNOS uncoupling, and the development of atherosclerosis: - Deficiency in L-arginine: Local deficit of L-arginine may derange eNOS, resulting in overproduction of superoxide radical instead of NO production [35, 60, 61].

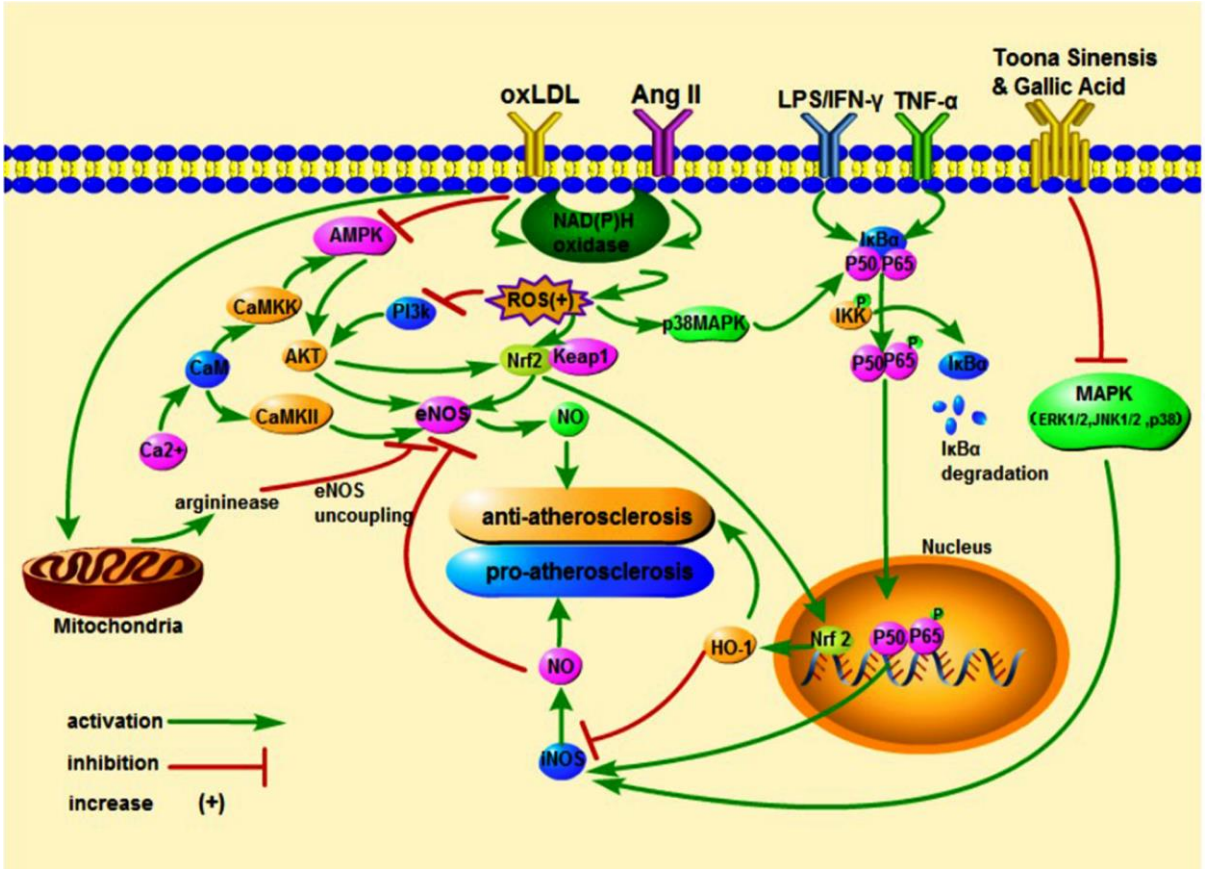

Fig. (1). The most important NO signaling pathways (eNOS/NO and iNOS/NO) in atherosclerosis. OxLDL: Oxidized low density lipoprotein; Ang II: Angiotensin II; LPS: Lipopolysaccharide; IFN-γ: Interferon-γ; TNF-α: Tumor necrosis factor-α; NAD(P)H oxidase: Nicotinamide adenine dinucleotidephosphateoxidase; ROS: Reactive oxygen species; PI3K: Phosphatidylinositol 3-kinase; AKT: Serine threonine protein kinase B; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide; AMPK: Adenosine monophosphate activated proteinkinase; CaMKII: Calmodulin dependent kinase II; CaMKKII: Calmodulindependent protein kinase kinaseII; Nrf2: Nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1; HO-1: Heme oxygenase-1; iNOS, Induciblenitric oxide synthase; IκB: Inhibitory subunit of NF-κB; IKK: IκB kinase complex; NF-κB: Nuclear factor-kappa B; MAPK: Mitogenactivated protein kinase; JNK: c-JunN-terminalkinase; ERK1/2: Extracellular regulated protein1/2 [1].

- Presence of endogenous competitive inhibitors of NOS, such as asymmetric dimethyl-l-arginine (ADMA), NGmonomethyl- L-arginine (L-NMMA) and symmetric dimethylarginine (SDMA) [62, 63]. All three inhibitors are synthesized *via* ethylation of L-arginine through protein arginine methyltransferases (PRMT) [64]. The action mechanisms of ADMA entail: competing with arginine

for the binding site of NOS to reduce NO formation, inhibiting eNOS and NO production and decreasing vascular compliance to enhance vascular resistance and limit blood flow [65]. SDMA acts by preventing L-arginine from entering cells [64] and L-NMMA competes with arginine for the binding site of NOS to decrease NO generation [64].

- Angiotensin II and enhancement or upregulation of arginase activity. Angiotensin II (Ang II) increases the activity of arginase *via* angiotensin II receptor type 1 (AT1) and reduces NO bioavailability [66]. Arginase (Arg) is an important enzyme in L-arginine catabolism and Arg I and Arg II directly compete with eNOS for the common substrate, L-arginine, in order to catalyze its conversion to ornithine and urea. It leads to the uncoupling of NOS and generation of ROS instead of NO production by consuming L-arginine and oxidizing BH4 cofactors [26, 67]. The small G protein RhoA and its effector Rho kinase (ROCK) play an important role in regulating arginase activity [15]. Thus, activation of pro-inflammatory pathways by Rho kinase (ROCK) activity in the endothelial cells leads to eNOS mRNA stability and decreases eNOS phyosphorylation as well as catalytic activity [68, 69].

- Higher levels of homocysteine (Hcy) cause NO pathway dysfunction and endothelial dysfunction through mechanisms such as oxidative stress, eNOS inhibition, NF-κB activation and inflammation. Hcy also increases ADMA generation and eNOS uncoupling by reducing the production of intracellular BH4 and thus decreases NO bioavailability [70].

3.4. Therapeutic possibilities of atherosclerosis and increasing NO bioavailability

The following represent some possibilities:
- Supplementation of the nitric oxide substrate, L-arginine
- Inhalation of nitric oxide
- Usage of NO donors and enhancers
- Vascular gene therapy of eNOS [5]
- Usage of antioxidants such as vitamins A and C, and medicinal plants [45].

3.4.1. Enhancers of NO Bioavailability and NO Donors

3.4.1.1. NO Donors

NO donors are pro-drugs that can exert their pharmacological and therapeutic effects after metabolism to nitric oxide.

3.4.1.2. Nitrovasodilators/Nitric Oxide Donors

Nitrovasodilators include sodium nitroprusside, amyl nitrite, glyceryl trinitrate, molsidomine and nitrosothiols. Nitric oxide donors include S-Nitroso-N-acetylpenicillamine (SNAP), 3-morpholinosydnonimine (SIN-1), nitroglycerin (NTG), sodium nitroprusside (SNP), isosorbide dinitrate (ISDN), amyl nitrite, isosorbide mononitrate (IS-5N), nicorandil and sydnonimines [5, 45, 71]. NO donors decrease the progression of lesion formation and platelet aggregating, LDL-oxidation and endothelial dysfunction [72].

3.4.1.3. NO Enhancers

Statins, L-arginine and tetrahydrobiopterin (BH4) are the most important NO enhancers. They increase the stability of eNOS messenger RNA. These enhancers reduce thrombosis, platelet aggregation, and expression of NAD(P)H oxidase subunits. Inhibition of superoxide anion generation by the endothelium and a shift in the balance between free radicals and NO are the other functions of NO enhancers [72]. NOS activators (including tetrahydrobiopterin, statins, eNOS transcriptional enhancers (AVE9488 and AVE3085), the protein kinase C inhibitor midostaurin,

trans-resveratrol, vanadate, betulinic acid and pentacyclic triteroenoids ursolic acid) have therapeutic properties in the treatment of arteriosclerotic vascular disease by increasing NOS expression and activity or decreasing NOS uncoupling *via* reduction of vascular and BH4 oxidation [15, 73].

3.4.2. Medicinal Plants and NO Bioavailability

Various medicinal plants and bioactive compounds including their secondary metabolites such as phenolic compounds, flavonoids, saponins, terpenoids and alkaloids are widely used for the enhancement of NO bioavailability and the treatment of atherosclerosis [74]. Different antioxidants increase NO production by protecting necessary cofactors such as BH4 from oxidation and they preserve NO activity and bioavailability [14]. Some medicinal plants enhance NO bioactivity and bioavailability *via* the nitrate-nitrite reduction pathway [14]. Also, antioxidants and polyphenols effectively reduce nitrite to NO conversion [14]. Data concerning the effective medicinal plants on the enhancement of NO production and ioavailability are summarized in Table **1**.

Sida cordifolia induces the release of NO through activation of the cardiac muscarinic system *via* the vagus nerve and stimulation of endothelial vascular M3 receptors [75]. *Euonymus alatus,* in combination with recombinant interferon-γ (rIFN- γ) induces NO production by peritoneal macrophages *via* the NF-κB signaling pathway and it synergistically increases the expression of iNOS protein [76]. *Ixeris dentate* and *Oldenlandia diffusa* in combination with recombinant interferon-γ (rIFN-γ) induces the production of NO and tumor necrosis factor-α (TNF-α) by macrophages through activation of the nuclear factor kappa B (NF-κB) signaling pathway. rIFN-γ and *Oldenlandia diffusa* on the other hand, synergistically
increase the expression of iNOS protein [77, 78]. *Centella asiatica* and *Rhinacanthus nasutus* in conjunction with LPS increase NO production and TNF-α by macrophages *via* gene expression and activation of the NF-κB signaling pathway [79]. *Cynara cardunculus* and *Thymus pulegioides* increase NO production by stimulating endothelial cells and cerebral cell membrane homogenates [80]. Inulin, an active component of *Chicorium intybus* root, with IFN-γ synergistically induces NO synthesis by RAW 264.7 cells through activation of protein kinase C (PKC)-α and protein tyrosine kinase (PTK) resulting in NF-κB activation [81]. *Echinacea purpurea* increases NO release from alveolar macrophages through the stimulation of lipopolysaccharides (LPS) [82, 83].

Acidic polysaccharides from *Phellinus linteus* induce NO and TNF-α production in peritoneal macrophages due to their immunomodulatory and anti-tumoricidal activities [84]. *Eucommia ulmoides* brings about the release of endothelium-derived hyperpolarizing factor (EDHF) and NO in smaller muscular vessels and endothelial cells. It can also inhibit adenosine 3',5'-cyclic monophosphate (cAMP) phosphodiesterase in vascular endothelial cells. Therefore, due to the accumulation of cAMP, the release of NO increases from the endothelium [85, 86]. *Trigonella foenum-graecum*, *Nigella sativa*, *Allium sativum*, and *Cannabis sativa* increase NO production because of the presence of diosgenin (25R-spirost-5-en-3β-ol)
in their extracts in the form of glucoside [87]. Methanol extracts of *Phyllunthus freternus, Triumfetta rhomboidae* and *Casuarina littorea* have nitric oxide scavenging activity due to the presence of tannins and flavonoids [88].

Fructus trichosanthis, *Resina olibani*, *Radix paeonia* rubra and *Borneolum syntheticum* have robust NO bioactivity by virtue of their ability to reduce nitrite to NO (NO generation from nitrite) and to restore NO homeostasis *via* nitrate–nitrite reduction pathways [89]. *Rumex acetosa* increases NO levels through the phosphorylation of eNOS by induction of the PI3K/AKT/eNOS pathway [90]. *Prunella vulgaris* induces eNOS and increases NO generation by activating the PI3K/AKT-mediated Nrf2 pathway [91].

Table 1. Effective medicinal plants on the enhancement of nitric oxide (NO) bioavailability.

Medicinal plants	Action mechanisms	References
Sida cordifolia	Cardiac muscarinic activation, direct activation of endothelial vascular muscarinic of M_3 receptors	75
Euonymus alatus	Nuclear factor kappa B (NF-κB) signaling pathway activation	76
Ixeris dentate		77, 78
Oldenlandia diffusa		
Centella asiatica	gene expression and NF-κB signaling pathway activation	79
Rhinacanthus nasutus		
Cynara cardunculus	Stimulation of endothelial cells and brain homogenate	80
Thymus pulegioides		
Chicorium intybus	Activation of protein kinase C (PKC)-α and protein tyrosine kinase (PTK)	81
Cocos nucifera	Immunomodulatory activity, induction of macrophages to NO generation, stimulation of LPS	93
Echinacea purpurea		82, 83
Phellinus linteus	Immunomodulatory and anti-tumoricidal activities, induction of macrophages to NO generation	84
Eucommia ulmoides	Inhibition cAMP phosphodiesterase and release of EDHF	85, 86
Trigonella foenum-graecum	Potent inhibition of lipid peroxidation, inhibition of superoxide production	87
Nigella sativa		
Allium sativum		
Cannabis sativa		
Phyllunthus freternus	Nitric oxide scavenging activity	88
Triumfetta rhomboidae		
Casuarina littorea		
Fructus trichosanthis	Reduction nitrite to NO via nitrate–nitrite reduction pathways and restoration of NO homeostasis	89
Borneolum Syntheticum		
Radix Paeonia Rubra		
Resina Olibani		
Rumex acetosa	Phosphorylation of eNOS by induction of PI3K/AKT/eNOS pathway	90
Prunella vulgaris	Induction of eNOS and activation of the PI3K/AKT-mediated Nrf2 pathway	91
Camelia japonica	Activation of eNOS via phosphorylation at Ser1179 and prevention of VSMCs proliferation and	92

	migration	
Radix Ginseng	Reduction nitrite to NO via nitrate–nitrite reduction pathways	89
Radix Notoginseng		89
Radix Salviae Miltiorrhizae		89, 95
Ginkgo biloba	eNOS gene expression, Phosphorylation of eNOS occurs at Ser 1177 through the phosphoinositide 3-kinase- (PI3K-) AKT pathway and decreasing AMPK dephosphorylation	97, 98
Angelica gigas	Direct phosphorylation of eNOS by the protein kinase Akt downstream of PI3K	99, 100
Paeonia lactiflora		
Lindera strichnifolia		
Caesalpinia sappan		
Cyperus rotundus		
Carthamus tinctorious		
Prunus persica		
Cinnamomum cassia		
Glycyrrhiza uralensis		
Panax ginseng radix	NO production via activation of K^+ channels	14
Cinnamomum cassia		
Sinomenum acutum		
Gypsum fibrosum		
Radix Puerariae	Induction of protein expression and activation of eNOS and through Akt/PKB phosphorylation	96
Allium sativum	Scavenging of free radicals, potent inhibition of lipid peroxidation, inhibition of superoxide production	101, 102

Camelia japonica stimulates NO production through the Akt pathway in endothelial cells and activation of eNOS *via* phosphorylation at Ser1179. *Camelia japonica* prevents VSMC proliferation and migration [92]. A polyphenolic-rich extract of *Cocos nucifera* can induce NO production by macrophages [93]. Effective components of *Nigella sativa* are thymoquinone and polyphenols that are NO production inducers [74, 87, 94].

Sanchi (*radix notoginseng*), Danshen Root (*radix salvia miltiorrhizae*) and Hongshen (*radix ginseng*) can relax blood vessels through the production of NO from nitrite [89]. Danshen can decrease cellular damage from ischemia and scavenge free radicals in ischemic diseases [95]. Salvianolic acid B (Sal B) and Tanshinone IIA (Tan IIA) in danshen mediate vasodilatation and stimulate eNOS phosphorylation [14]. *Radix ginseng* has reductase activity [89], whilst *Radix puerariae* and its major component puerarin increase serum nitrite concentrations by inducing protein expression and activating eNOS *via* Akt/PKB phosphorylation [96]. *Ginkgo biloba* causes endothelial nitric oxide (NO) production by enhancing endothelial nitric oxide synthase (eNOS) activity and eNOS gene expression. Phosphorylation of eNOS occurs at Ser 1177 *via* the phosphoinositide 3-kinase- (PI3K-) AKT pathway [97, 98]. Its extract decreases adenosine monophosphate activated protein kinase (AMPK) dephosphorylation and increases subsequently activated protein kinase C (PKC)-induced membrane subunits gp91 and p22 (phox) protein expression [98].

Dangkwisoo-San includes nine species of herbal plants (Angelica gigas, Paeonia lactiflora, Lindera strichnifolia, Caesalpinia sappan, Cyperus rotundus, Carthamus tinctorious, Prunus persica, Cinnamomum cassia, and Glycyrrhiza uralensis). All of these medicinal plants can produce NO *via* direct eNOS phosphorylation by protein kinase Akt downstream of PI3K such that eNOS improves cerebral blood flow [99, 100].

Mu-Fang-Ji-Tang contains four natural medicinal plants (*Sinomenum acutum*, *Cinnamomum cassia*, *Panax ginseng radix* and *Gypsum fibrosum*) that have protective effects against myocardial injury and heart failure *via* NO production and activation of K^+ channels [14].

Allium sativum (garlic) has antioxidant properties and enhances the production of nitric oxide along with free radical scavenging activity. It is a potent inhibitor not only of lipid peroxidation and superoxide production, but also xanthine oxidase, a source of superoxide important in vascular beds, which is all reflective of antiatherosclerotic activity [101, 102].

3.4.3. Bioactive Compounds

Data concerning the effective natural and bioactive compounds on the enhancement of NO production and bioavailability are summarized in Table **2**. Flavonoids (a large group of polyphenols) are important plant-derived compounds involved in cardiovascular prevention by reducing oxidative stress and increasing NO bioavailability [44]. Flavonoids are subclassified as flavonols such as quercitin (in tea, wine, red onions, cranberries, buckwheat, apples and beans), flavones such as apigenin (in bilberry, raspberry, strawberry, plum, cherry, blackberry, red pepper, and tomato skin) flavanols or flavan-3-ols such as catechins, epicatechins and their oligomers such as proanthocyanidins (in wine, apple juice, tea and cocoa), isoflavones such as genistein (in soy, legumes), and anthocyanins [103, 104].

Polyphenols induce NO formation through the activation of the phosphatidylinositol 3-kinase/Akt pathway leading to eNOS activation [44]. Dietary polyphenol consumption (from wine, cocoa, or tea, flavonoid-rich cocoa and beverage) protects NO against oxidants and increases NO bioavailability in human plasma [44]. Polyphenols of black tea increase eNOS activity *via* p38 MAPK dependent phosphorylation in aortic endothelial cells [105].

Stilbenoids such as resveratrol induce platelet NO production through inhibition of p38 MAPK, NADPH oxidases, and superoxide formation. It attenuates eNOS uncoupling *via* reduction of Larginine levels, oxidative stress and enhances levels of BH4 [106, 107]. Catechins in green tea activate eNOS by phosphorylation at Ser1179 and dephosphorylation at Thr495 in a PKA-Akt dependent manner [108, 109]. Quercitin induces NO production *via* inhibition/downregulation of NADPH oxidase (down-regulation of aortic p47phox, a regulatory subunit of NADPH oxidase) and restoration of NOS regulation [110,111]. Genistein (a soy isoflavone) enhances eNOS activity *via* inhibition of NADPH oxidase and reduction of superoxide formation [112]. Apigenin and curcumin increase NO bioavailability by decreasing oxidative stress [15]. Saponins (plant glycosides) from *Ginseng* stimulate NO release from vascular endothelial cells *via* activation of K+ channels [113, 114]. Bromelain increases inflammatory mediators such as IFN-γ-mediated nitric oxide (NO) and TNF-α production in macrophages [115]. Myricitrin, Epigallocatechin Gallate (EGCG) and Salidroside (*Rhodiola rosea*) decrease atherosclerotic plaques by activating the PI3K/AKT/eNOS/NO pathway *via* phosphorylation of threonine 308 and serine 473 and AKT activation in order to promote eNOS for NO production [116-118]. B-carotene and Betulinic acid induce NO production *via* AMPK, eNOS phosphorylation and Ca (2^+) /CaMKKII / eNOS (+) or the CaMKK/AMPK/AKT/eNOS/NO signaling pathway [119, 120].

Table 2. Effective natural and bioactive compounds on enhancement of nitric oxide (NO).

Active compounds	Action mechanisms	References
Resveratrol	Inhibition of p38 MAPK, NADPH oxidases, and superoxide formation, enhancement levels of BH_4, inhibition of eNOS uncoupling	106, 107
Catechin	Activation of eNOS by phosphorylation at Ser1179 and dephosphorylation at Thr495 in a PKA-Akt dependent manner	108, 109
Quercitin	Inhibition/downregulation of NADPH oxidase	110, 111
Genistein	Enhancement of eNOS activity via inhibition of NADPH oxidase and reduction of superoxide formation	112
Apigenin	Decreasing oxidative stress	15
Curcumin		
Saponin	activation of K^+ channels	113, 114
Bromelain	Increasing inflammatory mediators such as IFN-γ-mediated nitric oxide (NO) and TNF-α production	115
Inulin	Activation of protein kinase C (PKC)-α and protein tyrosine kinase (PTK)	81
Myricitrin	Activating PI3K/AKT/eNOS/NO pathway via phosphorylation on threonine 308 and serine 473, and AKT activation	116
Salidroside		117
Epigallocatechin Gallate (EGCG)		118
B-carotene	eNOS phosphorylation and Ca (2 +) / CaMKKII / eNOS (+) or CaMKK/AMPK/AKT/eNOS/ NO signaling pathway	119
Betulinic acid		120

CONCLUSION

Atherosclerosis is a leading cause of mortality and morbidity globally. NO has vasculoprotective actions such as inhibition of leukocyte adhesion, platelet aggregation and prevention of VSMC proliferation. Reduced NO bioavailability and endothelial dysfunction are the early markers of atherosclerosis. Loss of NO activity and its bioavailability may lead to detrimental vascular effects such as impairment of endothelial function, increased activity and adherence of platelets, enhanced smooth muscle cell proliferation and atheromatous plaque formation, inflammatory cells, myocardial infarction, stroke and peripheral ischaemia, increased adhesion of leukocytes, increased generation

of $O2^-$ radicals, oxidation of LDL along with subsequent foam cell formation and atherosclerosis progression. The strategies to increase NO bioavailability are beneficial in the treatment of atherosclerosis. Medicinal plants and their secondary metabolites are good therapeutic options for the enhancement of NO bioavailability through increasing endothelial production of NO, activation of protein kinase C (PKC)-α and protein tyrosine kinase (PTK), Nuclear factor kappa B (NF-κB) signaling pathway activation and decreasing of oxidative stress. Therefore, they might be considered as prospective safe candidates for atherosclerosis preventative therapy.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Wang XF, Ye ZX, Chen JY, *et al.* Roles of nitric oxide signaling pathway in atherosclerosis. Atheroscler Open Access 31

[2] Charakida M, Deanfield JE, Halcox JP. The role of nitric oxide in early atherosclerosis. Eur J Clin Pharmacol 2006; 62: 69-78. http://dx.doi.org/10.1007/s00228-005-0007-9

[3] Chen JY, Ye ZX, Wang XF, *et al.* Nitric oxide bioavailability dysfunction involves in atherosclerosis. Biomed Pharmacother 2018; 97: 423-8. http://dx.doi.org/10.1016/j.biopha.2017.10.122 PMID: 29091892

[4] Matthys KE, Bult H. Nitric oxide function in atherosclerosis. Mediators Inflamm 1997; 6(1): 3-21. http://dx.doi.org/10.1080/09629359791875 PMID: 18472828

[5] Vallance P, Chan N. Endothelial function and nitric oxide: clinical relevance. Heart 2001; 85(3): 342-50. http://dx.doi.org/10.1136/heart.85.3.342 PMID: 11179281

[6] Stapleton PA, Goodwill AG, James ME, Brock RW, Frisbee JC. Hypercholesterolemia and microvascular dysfunction: interventional strategies. J Inflamm (Lond) 2010; 7: 54. http://dx.doi.org/10.1186/1476-9255-7-54 PMID: 21087503

[7] Miller MR, Megson IL. Recent developments in nitric oxide donor drugs. Br J Pharmacol 2007; 151(3): 305-21. http://dx.doi.org/10.1038/sj.bjp.0707224 PMID: 17401442

[8] Tatematsu S, Wakino S, Kanda T, *et al.* Role of nitric oxideproducing and -degrading pathways in coronary endothelial dysfunction in chronic kidney disease. J Am Soc Nephrol 2007; 18(3): 741-9. http://dx.doi.org/10.1681/ASN.2006040367 PMID: 17267746

[9] Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327(6122): 524-6. http://dx.doi.org/10.1038/327524a0 PMID: 3495737

[10] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288(5789): 373-6. http://dx.doi.org/10.1038/288373a0 PMID: 6253831

[11] Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 1987; 84(24): 9265-9. http://dx.doi.org/10.1073/pnas.84.24.9265 PMID: 2827174

[12] Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988; 333(6174): 664-6. http://dx.doi.org/10.1038/333664a0 PMID: 3131684

[13] Marletta MA. Nitric oxide synthase: aspects concerning structure and catalysis. Cell 1994; 78(6): 927-30. http://dx.doi.org/10.1016/0092-8674(94)90268-2 PMID: 7522970

[14] Jiang H, Torregrossa AC, Parthasarathy DK, Bryan NS. Natural product nitric oxide chemistry: new activity of old medicines. Evid Based Complement Alternat Med 2012; 2012873210 http://dx.doi.org/10.1155/2012/873210 PMID: 22548122

[15] Forte M, Conti V, Damato A, *et al.* Targeting nitric oxide with natural derived compounds as a therapeutic strategy in vascular diseases. Oxid Med Cell Longev 2016; 20167364138 http://dx.doi.org/10.1155/2016/7364138 PMID: 27651855

[16] Tsutsui M, Shimokawa H, Otsuji Y, Ueta Y, Sasaguri Y, Yanagihara N. Nitric oxide synthases and cardiovascular diseases: insights from genetically modified mice. Circ J 2009; 73(6): 986-93. http://dx.doi.org/10.1253/circj.CJ-09-0208 PMID: 19430166

[17] Tsutsui M, Shimokawa H, Tanimoto A, Yanagihara N, Tamura M, Otsuj Y. Roles of Nitric Oxide Synthases in Arteriosclerotic Vascular Disease: Insights from Murine Genetic Models. J Clin Exp Cardiol 2014; 5: 2-7.

[18] Xie Q-W, Cho HJ, Calaycay J, *et al.* Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science 1992; 256(5054): 225-8. http://dx.doi.org/10.1126/science.1373522 PMID: 1373522

[19] Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. Cell 1994; 78(6): 915-8. http://dx.doi.org/10.1016/0092-8674(94)90266-6 PMID: 7522969

[20] Costa ED, Rezende BA, Cortes SF, Lemos VS. Neuronal nitric oxide synthase in vascular physiology and diseases. Front Physiol 2016; 7: 206-14. http://dx.doi.org/10.3389/fphys.2016.00206 PMID: 27313545

[21] Rafikov R, Fonseca FV, Kumar S, *et al.* eNOS activation and NO function: structural motifs responsible for the posttranslational control of endothelial nitric oxide synthase activity. J Endocrinol 2011; 210(3): 271-84. http://dx.doi.org/10.1530/JOE-11-0083 PMID: 21642378

[22] Atochin DN, Huang PL. Endothelial nitric oxide synthase transgenic models of endothelial dysfunction. Pflugers Arch 2010; 460(6): 965-74. http://dx.doi.org/10.1007/s00424-010-0867-4 PMID: 20697735

[23] Böger RH, Bode-Böger SM, Frölich JC. The L-arginine-nitric oxide pathway: role in atherosclerosis and therapeutic implications. Atherosclerosis 1996; 127(1): 1-11. http://dx.doi.org/10.1016/S0021-9150(96)05953-9 PMID: 9006798

[24] Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. J Cardiovasc Pharmacol 1999; 34(6): 879-86. http://dx.doi.org/10.1097/00005344-199912000-00016 PMID: 10598133

[25] Clancy RM, Leszczynska-Piziak J, Abramson SB. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production *via* a direct action on the NADPH oxidase. J Clin Invest 1992; 90(3): 1116-21. http://dx.doi.org/10.1172/JCI115929 PMID: 1325992

[26] Gliozzi M, Scicchitano M, Bosco F, *et al.* Modulation of Nitric Oxide Synthases by Oxidized LDLs: Role in Vascular Inflammation and Atherosclerosis Development. Int J Mol Sci 2019; 20(13): 3294-310. http://dx.doi.org/10.3390/ijms20133294 PMID: 31277498

[27] Sukhovershin RA, Yepuri G, Ghebremariam YT. Endotheliumderived nitric oxide as an antiatherogenic mechanism: implications for therapy. Methodist DeBakey Cardiovasc J 2015; 11(3): 166-71. http://dx.doi.org/10.14797/mdcj-11-3-166 PMID: 26634024

[28] Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991; 88(11): 4651-5. http://dx.doi.org/10.1073/pnas.88.11.4651 PMID: 1675786

[29] Gauthier TW, Davenpeck KL, Lefer AM. Nitric oxide attenuates leukocyte-endothelial interaction *via* P-selectin in splanchnic ischemia-reperfusion. Am J Physiol 1994; 267(4 Pt 1): G562-8. PMID: 7524346

[30] Tsao PS, McEvoy LM, Drexler H, Butcher EC, Cooke JP. Enhanced endothelial adhesiveness in hypercholesterolemia is attenuated by L-arginine. Circulation 1994; 89(5): 2176-82. http://dx.doi.org/10.1161/01.CIR.89.5.2176 PMID: 8181143

[31] von der Leyen HE, Gibbons GH, Morishita R, *et al*. Gene therapy inhibiting neointimal vascular lesion: *in vivo* transfer of endothelial cell nitric oxide synthase gene. Proc Natl Acad Sci USA 1995; 92(4): 1137-41. http://dx.doi.org/10.1073/pnas.92.4.1137 PMID: 7532305

[32] Ellenby MI, Ernst CB, Carretero OA, Scicli AG. Role of nitric oxide in the effect of blood flow on neointima formation. J Vasc Surg 1996; 23(2): 314-22. http://dx.doi.org/10.1016/S0741-5214(96)70276-8 PMID: 8637109

[33] Farhy RD, Carretero OA, Ho KL, Scicli AG. Role of kinins and nitric oxide in the effects of angiotensin converting enzyme inhibitors on neointima formation. Circ Res 1993; 72(6): 1202-10. http://dx.doi.org/10.1161/01.RES.72.6.1202 PMID: 7684331

[34] Shaw CA, Megson IL, Rossi AG. Apoptosis and atherosclerosis: The role of nitric oxide. Antiinflamm Antiallergy Agents Med Chem 2006; 5: 27-33. http://dx.doi.org/10.2174/187152306775537300

[35] Napoli C, de Nigris F, Williams-Ignarro S, Pignalosa O, Sica V, Ignarro LJ. Nitric oxide and atherosclerosis: an update. Nitric Oxide 2006; 15(4): 265-79. http://dx.doi.org/10.1016/j.niox.2006.03.011 PMID: 16684613

[36] Muller G, Morawietz H. Nitric oxide, NAD(P)H oxidase, and atherosclerosis. Antioxid Redox Signal 2009; 11(7): 1711-31. http://dx.doi.org/10.1089/ars.2008.2403 PMID: 19257809

[37] Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 2006; 113(13): 1708-14. http://dx.doi.org/10.1161/CIRCULATIONAHA.105.602532 PMID: 16585403

[38] Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. Circ J 2009; 73(3): 411-8. http://dx.doi.org/10.1253/circj.CJ-08-1102 PMID: 19194043

[39] Parthasarathy S, Santanam N. Mechanisms of oxidation, antioxidants, and atherosclerosis. Curr Opin Lipidol 1994; 5(5): 371-5. http://dx.doi.org/10.1097/00041433-199410000-00009 PMID: 7858912

[40] Malekmohammad K, Rafieian-Kopaei M, Sardari S, Sewell RD. Toxicological effects of *Mentha* x *piperita* (peppermint): a review. Toxin Rev 2019: 1-15. http://dx.doi.org/10.1080/15569543.2019.1647545

[41] Katz SD, Biasucci L, Sabba C, *et al.* Impaired endothelium mediated vasodilation in the peripheral vasculature of patients with congestive heart failure. J Am Coll Cardiol 1992; 19(5): 918-25. http://dx.doi.org/10.1016/0735-1097(92)90271-N PMID: 1552112

[42] Ludmer PL, Selwyn AP, Shook TL, *et al.* Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986; 315(17): 1046-51. http://dx.doi.org/10.1056/NEJM198610233151702 PMID: 3093861

[43] Félétou M, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). Am J Physiol Heart Circ Physiol 2006; 291(3): H985-H1002. http://dx.doi.org/10.1152/ajpheart.00292.2006 PMID: 16632549

[44] Grassi D, Desideri G, Ferri C. Flavonoids: antioxidants against atherosclerosis. Nutrients 2010; 2(8): 889-902. http://dx.doi.org/10.3390/nu2080889 PMID: 22254061

[45] Napoli C, Ignarro LJ. Nitric oxide and atherosclerosis. Nitric Oxide 2001; 5(2): 88-97. http://dx.doi.org/10.1006/niox.2001.0337 PMID: 11292358

[46] Soydinç S, Çelik A, Demiryürek S, Davutoğlu V, Tarakçıoğlu M,Aksoy M. The relationship between oxidative stress, nitric oxide and coronary artery disease. Eur J Gen Med 2007; 4: 62-6.

[47] Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997; 272(34): 20963-6. http://dx.doi.org/10.1074/jbc.272.34.20963 PMID: 9261091

[48] Li H, Förstermann U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. Curr Opin Pharmacol 2013; 13(2): 161-7. http://dx.doi.org/10.1016/j.coph.2013.01.006 PMID: 23395155

[49] Vásquez-Vivar J, Kalyanaraman B, Martásek P, *et al.* Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc Natl Acad Sci USA 1998; 95(16): 9220-5. http://dx.doi.org/10.1073/pnas.95.16.9220 PMID: 9689061

[50] Wang W, Wang S, Yan L, *et al.* Superoxide production and reactive oxygen species signaling by endothelial nitric-oxide synthase. J Biol Chem 2000; 275(22): 16899-903. http://dx.doi.org/10.1074/jbc.M000301200 PMID: 10747895

[51] Goncharov NV, Avdonin PV, Nadeev AD, Zharkikh IL, Jenkins RO, Jenkins R. Reactive oxygen species in pathogenesis of atherosclerosis. Curr Pharm Des 2015; 21(9): 1134-46. http://dx.doi.org/10.2174/1381612820666141014142557 PMID: 25312724

[52] Malekmohammad K, Sewell RDE, Rafieian-Kopaei M. Antioxidants and Atherosclerosis: Mechanistic Aspects. Biomolecules 2019; 9(8): 301-19. http://dx.doi.org/10.3390/biom9080301 PMID: 31349600

[53] Landmesser U, Dikalov S, Price SR, *et al.* Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003; 111(8): 1201-9. http://dx.doi.org/10.1172/JCI200314172 PMID: 12697739

[54] Bec N, Gorren ACF, Voelker C, Mayer B, Lange R. Reaction of neuronal nitric oxide synthase with oxygen at low temperature. J Biol Chem 1998; 273: 13502-8. http://dx.doi.org/10.1074/jbc.273.22.13502 PMID: 9593685

[55] Crabtree MJ, Smith CL, Lam G, Goligorsky MS, Gross SS. Ratio of 5,6,7,8-tetrahydrobiopterin to 7,8-dihydrobiopterin in endothelial cells determines glucose-elicited changes in NO vs. superoxide production by eNOS. Am J Physiol Heart Circ Physiol 2008; 294(4): H1530-40. http://dx.doi.org/10.1152/ajpheart.00823.2007 PMID: 18192221

[56] Pong T, Huang PL. Effects of Nitric Oxide on Atherosclerosis. Atherosclerosis 2015: 355-64.

[57] Cheng C, van Haperen R, de Waard M, *et al.* Shear stress affects the intracellular distribution of eNOS: direct demonstration by a novel *in vivo* technique. Blood 2005; 106(12): 3691-8. http://dx.doi.org/10.1182/blood-2005-06-2326 PMID: 16105973

[58] Hishikawa K, Lüscher TF. Pulsatile stretch stimulates superoxide production in human aortic endothelial cells. Circulation 1997; 96(10): 3610-6. http://dx.doi.org/10.1161/01.CIR.96.10.3610 PMID: 9396462

[59] Duerrschmidt N, Stielow C, Muller G, Pagano PJ, Morawietz H. NO-mediated regulation of NAD(P)H oxidase by laminar shear stress in human endothelial cells. J Physiol 2006; 576(Pt 2): 557-67. http://dx.doi.org/10.1113/jphysiol.2006.111070 PMID: 16873416

[60] Vergnani L, Hatrik S, Ricci F, *et al.* Effect of native and oxidized low-density lipoprotein on endothelial nitric oxide and superoxide production: key role of L-arginine availability. Circulation 2000; 101(11): 1261-6. http://dx.doi.org/10.1161/01.CIR.101.11.1261 PMID: 10725285

[61] Ignarro LJ, Napoli C. Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis. Curr Atheroscler Rep 2004; 6(4): 281-7. http://dx.doi.org/10.1007/s11883-004-0059-9 PMID: 15191702

[62] Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet 1989;2(8670): 997-1000. http://dx.doi.org/10.1016/S0140-6736(89)91013-1 PMID: 2572793

[63] Rochette L, Lorin J, Zeller M, *et al.* Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: possible therapeutic targets? Pharmacol Ther 2013; 140(3): 239-57. http://dx.doi.org/10.1016/j.pharmthera.2013.07.004 PMID: 23859953

[64] Bartnicki P, Kowalczyk M, Franczyk-Skóra B, Baj Z, Rysz J. Evaluation of endothelial (dys) function, left ventricular structure and function in patients with chronic kidney disease. Curr Vasc Pharmacol 2016; 14(4): 360-7. http://dx.doi.org/10.2174/1570161114666160112142403 PMID: 26759218

[65] Tran CT, Leiper JM, Vallance P. The DDAH/ADMA/NOS pathway. Atheroscler Suppl 2003; 4(4): 33-40. http://dx.doi.org/10.1016/S1567-5688(03)00032-1 PMID: 14664901

[66] Shatanawi A, Lemtalsi T, Yao L, Patel C, Caldwell RB, Caldwell RW. Angiotensin II limits NO production by upregulating arginase through a p38 MAPK-ATF-2 pathway. Eur J Pharmacol 2015; 746: 106-14. http://dx.doi.org/10.1016/j.ejphar.2014.10.042 PMID: 5446432

[67] Lee J, Bae EH, Ma SK, Kim SW. Altered nitric oxide system in cardiovascular and renal diseases. Chonnam Med J 2016; 52(2): 81-90. http://dx.doi.org/10.4068/cmj.2016.52.2.81 PMID: 27231671

[68] Satoh K, Fukumoto Y, Shimokawa H. Rho-kinase: important new therapeutic target in cardiovascular diseases. Am J Physiol Heart Circ Physiol 2011; 301(2): H287-96. http://dx.doi.org/10.1152/ajpheart.00327.2011 PMID: 21622831

[69] Batchelor TJ, Sadaba JR, Ishola A, Pacaud P, Munsch CM, Beech DJ. Rho-kinase inhibitors prevent agonist-induced vasospasm in human internal mammary artery. Br J Pharmacol 2001; 132(1): 302-8. http://dx.doi.org/10.1038/sj.bjp.0703809 PMID: 11156590

[70] Chen Y, Zhao S, Wang Y, *et al.* Homocysteine reduces protein S nitrosylation in endothelium. Int J Mol Med 2014; 34(5): 1277-85. http://dx.doi.org/10.3892/ijmm.2014.1920 PMID: 25189662

[71] Bryan NS. Nitric oxide enhancement strategies. Future Sci OA 2015; 1(1): FSO48. http://dx.doi.org/10.4155/fso.15.48 PMID: 28031863

[72] Herman AG, Moncada S. Therapeutic potential of nitric oxide donors in the prevention and treatment of atherosclerosis. Eur Heart J 2005; 26(19): 1945-55. http://dx.doi.org/10.1093/eurheartj/ehi333 PMID: 15911567

[73] Papapetropoulos A, Fulton D, Lin MI, *et al.* Vanadate is a potent activator of endothelial nitric-oxide synthase: evidence for the role of the serine/threonine kinase Akt and the 90-kDa heat shock protein. Mol Pharmacol 2004; 65(2): 407-15. http://dx.doi.org/10.1124/mol.65.2.407 PMID: 14742683

[74] Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC Complement Altern Med 2015; 15: 159-69. http://dx.doi.org/10.1186/s12906-015-0669-5 PMID: 26014115

[75] Medeiros IA, Santos MRV, Nascimento NMS, Duarte JC. Cardiovascular effects of Sida cordifolia leaves extract in rats. Fitoterapia 2006; 77(1): 19-27. http://dx.doi.org/10.1016/j.fitote.2005.06.003 PMID: 16257496

[76] Chung HS, Jeong HJ, Kim JS, *et al.* Activation of inducible nitric oxide synthase by Euonymus alatus in mouse peritoneal macrophages. Clin Chim Acta 2002; 318(1-2): 113-20. http://dx.doi.org/10.1016/S0009-8981(01)00808-7 PMID: 11880120

[77] Chung HS, Jeong HJ, Han MJ, *et al.* Nitric oxide and tumor necrosis factor-alpha production by Ixeris dentata in mouse peritoneal macrophages. J Ethnopharmacol 2002; 82(2-3): 217-22. http://dx.doi.org/10.1016/S0378-8741(02)00188-5 PMID: 12241998

[78] Chung HS, Jeong HJ, Hong SH, *et al.* Induction of nitric oxide synthase by Oldenlandia diffusa in mouse peritoneal macrophages. Biol Pharm Bull 2002; 25(9): 1142-6. http://dx.doi.org/10.1248/bpb.25.1142 PMID: 12230105

[79] Punturee K, Wild CP, Vinitketkumneun U. Thai medicinal plants modulate nitric oxide and tumor necrosis factor-α in J774.2 mouse macrophages. J Ethnopharmacol 2004; 95(2-3): 183-9. http://dx.doi.org/10.1016/j.jep.2004.06.019 PMID: 15507334

[80] Grande S, Bogani P, de Saizieu A, Schueler G, Galli C, Visioli F. Vasomodulating potential of mediterranean wild plant extracts. J Agric Food Chem 2004; 52(16): 5021-6. http://dx.doi.org/10.1021/jf049436e PMID: 15291469

[81] Koo HN, Hong SH, Seo HG, *et al.* Inulin stimulates NO synthesis *via* activation of PKC-α and protein tyrosine kinase, resulting in the activation of NF-kappaB by IFN-γ-primed RAW 264.7 cells. J Nutr Biochem 2003; 14(10): 598-605. http://dx.doi.org/10.1016/j.jnutbio.2003.07.002 PMID: 14559111

[82] Rininger JA, Kickner S, Chigurupati P, McLean A, Franck Z. Immunopharmacological activity of Echinacea preparations following simulated digestion on murine macrophages and human peripheral blood mononuclear cells. J Leukoc Biol 2000; 68(4): 503-10. PMID: 11037971

[83] Goel V, Chang C, Slama J, *et al.* Echinacea stimulates macrophage function in the lung and spleen of normal rats. J Nutr Biochem 2002; 13(8): 487-92. http://dx.doi.org/10.1016/S0955-2863(02)00190-0 PMID: 12165361

[84] Kim GY, Choi GS, Lee SH, Park YM. Acidic polysaccharide isolated from Phellinus linteus enhances through the up-regulation of nitric oxide and tumor necrosis factor-α from peritoneal macrophages. J Ethnopharmacol 2004; 95(1): 69-76. http://dx.doi.org/10.1016/j.jep.2004.06.024 PMID: 15374609

[85] Kwan CY, Zhang WB, Deyama T, Nishibe S. Endotheliumdependent vascular relaxation induced by Eucommia ulmoides Oliv. bark extract is mediated by NO and EDHF in small vessels. Naunyn Schmiedebergs Arch Pharmacol 2004; 369(2): 206-11. http://dx.doi.org/10.1007/s00210-003-0822-4 PMID: 14673511

[86] Zheng XF, Kwan CY, Daniel EE. β-Adrenoceptor activates endothelium-dependent release of nitric oxide in rat aorta. Zhongguo Yao Li Xue Bao 1995; 16(5): 385-90. PMID: 8701749

[87] Mohebbati R, Iranmanesh M, Beheshti F, *et al.* The Effect of Some Herbal Extracts on Nitric Oxide Production in Endothelial Cells 3T3 Cell Line. Iran J Pharm Sci 2016; 12: 1-10.

[88] Parul R, Kundu SK, Saha P. *In vitro* nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. The pharma innovation 2013; 1: 83-90.

[89] Tang Y, Garg H, Geng YJ, Bryan NS. Nitric oxide bioactivity of traditional Chinese medicines used for cardiovascular indications. Free Radic Biol Med 2009; 47(6): 835-40. http://dx.doi.org/10.1016/j.freeradbiomed.2009.06.024 PMID: 19545619

[90] Sun YY, Su XH, Jin JY, *et al.* Rumex acetosa L. induces vasorelaxation in rat aorta *via* activation of PI3-kinase/Akt- AND $Ca^{(2+)}$-eNOS-NO signaling in endothelial cells. J Physiol Pharmacol 2015; 66(6): 907-15. PMID: 26769840

[91] Hwang SM, Lee YJ, Yoon JJ, *et al.* Prunella vulgaris suppresses HG-induced vascular inflammation *via* Nrf2/HO-1/eNOS activation. Int J Mol Sci 2012; 13(1): 1258-68. http://dx.doi.org/10.3390/ijms13011258 PMID: 22312316

[92] Park SH, Shim BS, Yoon JS, *et al.* Vascular protective effect of an ethanol extract of Camellia japonica fruit: endothelium-dependent relaxation of coronary artery and reduction of smooth muscle cell migration. Oxid Med Cell Longev 2016: 2016-25. PMID: 26697138

[93] Mendonça-Filho RR, Rodrigues IA, Alviano DS, *et al.* Leishmanicidal activity of polyphenolic-rich extract from husk fiber of Cocos nucifera Linn. (Palmae). Res Microbiol 2004; 155(3): 136-43. http://dx.doi.org/10.1016/j.resmic.2003.12.001 PMID: 15059625

[94] Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. Phytother Res 2003; 17(4): 299-305. http://dx.doi.org/10.1002/ptr.1309 PMID: 12722128

[95] Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 2005; 45(12): 1345-59. http://dx.doi.org/10.1177/0091270005282630 PMID: 16291709

[96] Zhang SY, Chen G, Wei PF, *et al.* The effect of puerarin on serum nitric oxide concentration and myocardial eNOS expression in rats with myocardial infarction. J Asian Nat Prod Res 2008; 10(3-4): 373-81. http://dx.doi.org/10.1080/10286020801892250 PMID: 18348063

[97] Koltermann A, Hartkorn A, Koch E, Fürst R, Vollmar AM, Zahler S. Ginkgo biloba extract EGb 761 increases endothelial nitric oxide production *in vitro* and *in vivo*. Cell Mol Life Sci 2007; 64(13): 1715-22. http://dx.doi.org/10.1007/s00018-007-7085-z PMID: 17497242

[98] Ou HC, Hsieh YL, Yang NC, *et al.* Ginkgo biloba extract attenuates oxLDL-induced endothelial dysfunction *via* an AMPKdependent mechanism. J Appl Physiol 2013; 114(2): 274-85. http://dx.doi.org/10.1152/japplphysiol.00367.2012 PMID: 23195633

[99] Kim JH, Park SH, Kim YW, *et al.* The traditional herbal medicine, Dangkwisoo-San, prevents cerebral ischemic injury through nitric oxide-dependent mechanisms. Evid Based Complement Alternat Med 2011; 2011718302 http://dx.doi.org/10.1155/2011/718302 PMID: 21423636

[100] Jahan N, Rahman K, Ali S. Cardioprotective and antilipidemic potential of cyperus rotundus in chemically induced cardiotoxicity. Int J Agric Biol 2012; 14: 989-92.

[101] Maslin DJ, Brown CA, Das I, Zhang XH. Nitric oxide--a mediator of the effects of garlic? Biochem Soc Trans 1997; 25(3): 408S. http://dx.doi.org/10.1042/bst025408s PMID: 9388638

[102] Sooranna SR, Hirani J, Das I. Garlic can induce both GTP cyclohydrolase and nitric oxide synthase activity in choriocarcinoma cells. Biochem Soc Trans 1995; 23(4): 543S. http://dx.doi.org/10.1042/bst023543s PMID: 8654728

[103] Grassi D, Desideri G, Croce G, Tiberti S, Aggio A, Ferri C. Flavonoids, vascular function and cardiovascular protection. Curr Pharm Des 2009; 15(10): 1072-84. http://dx.doi.org/10.2174/138161209787846982 PMID: 19355949

[104] Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79(5): 727-47. http://dx.doi.org/10.1093/ajcn/79.5.727 PMID: 15113710

[105] Anter E, Thomas SR, Schulz E, Shapira OM, Vita JA, Keaney JF Jr. Activation of endothelial nitric-oxide synthase by the p38 MAPK in response to black tea polyphenols. J Biol Chem 2004; 279(45): 46637-43. http://dx.doi.org/10.1074/jbc.M405547200 PMID: 15333638

[106] Gresele P, Pignatelli P, Guglielmini G, *et al.* Resveratrol, at concentrations attainable with moderate wine consumption, stimulates human platelet nitric oxide production. J Nutr 2008; 138(9): 1602-8. http://dx.doi.org/10.1093/jn/138.9.1602 PMID: 18716157

[107] Bhatt SR, Lokhandwala MF, Banday AA. Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. Eur J Pharmacol 2011; 667(1-3): 258-64. http://dx.doi.org/10.1016/j.ejphar.2011.05.026 PMID: 21640096

[108] Lorenz M, Wessler S, Follmann E, *et al.* A constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-, cAMP-dependent protein kinase-,

and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. J Biol Chem 2004; 279(7): 6190-5. http://dx.doi.org/10.1074/jbc.M309114200 PMID: 14645258

[109] Kim JA, Formoso G, Li Y, *et al.* Epigallocatechin gallate, a green tea polyphenol, mediates NO-dependent vasodilation using signaling pathways in vascular endothelium requiring reactive oxygen species and Fyn. J Biol Chem 2007; 282(18): 13736-45. http://dx.doi.org/10.1074/jbc.M609725200 PMID: 17363366

[110] Zhang Y, Huang C, Liu S, *et al.* Effects of quercetin on intracavernous pressure and expression of nitrogen synthase isoforms in arterial erectile dysfunction rat model. Int J Clin Exp Med 2015; 8(5): 7599-605. PMID: 26221305

[111] Perez-Vizcaino F, Duarte J, Jimenez R, Santos-Buelga C, Osuna A. Antihypertensive effects of the flavonoid quercetin. Pharmacol Rep 2009; 61(1): 67-75. http://dx.doi.org/10.1016/S1734-1140(09)70008-8 PMID: 19307694

[112] Vera R, Galisteo M, Villar IC, *et al.* Soy isoflavones improve endothelial function in spontaneously hypertensive rats in an estrogen independent manner: role of nitric-oxide synthase, superoxide, and cyclooxygenase metabolites. J Pharmacol Exp Ther 2005; 314(3): 1300-9. http://dx.doi.org/10.1124/jpet.105.085530 PMID: 15958720

[113] Chen X. Cardiovascular protection by ginsenosides and their nitric oxide releasing action. Clin Exp Pharmacol Physiol 1996; 23(8): 728-32. http://dx.doi.org/10.1111/j.1440-1681.1996.tb01767.x PMID: 8886498

[114] Kim ND, Kang SY, Kim MJ, Park JH, Schini-Kerth VB. The ginsenoside Rg3 evokes endothelium-independent relaxation in rat aortic rings: role of K^+ channels. Eur J Pharmacol 1999; 367(1): 51-7. http://dx.doi.org/10.1016/S0014-2999(98)00899-1 PMID: 10082264

[115] Engwerda CR, Andrew D, Murphy M, Mynott TL. Bromelain activates murine macrophages and natural killer cells *in vitro*. Cell Immunol 2001; 210(1): 5-10. http://dx.doi.org/10.1006/cimm.2001.1793 PMID: 11485347

[116] Qin M, Luo Y, Meng XB, *et al.* Myricitrin attenuates endothelial cell apoptosis to prevent atherosclerosis: An insight into PI3K/Akt activation and STAT3 signaling pathways. Vascul Pharmacol 2015; 70: 23-34. http://dx.doi.org/10.1016/j.vph.2015.03.002 PMID: 25849952

[117] Xing SS, Yang XY, Zheng T, *et al.* Salidroside improves endothelial function and alleviates atherosclerosis by activating a mitochondria-related AMPK/PI3K/Akt/eNOS pathway. Vascul Pharmacol 2015; 72: 141-52. http://dx.doi.org/10.1016/j.vph.2015.07.004 PMID: 26187353

[118] Liu S, Sun Z, Chu P, *et al.* EGCG protects against homocysteineinduced human umbilical vein endothelial cells apoptosis by modulating mitochondrial-dependent apoptotic signaling and PI3K/Akt/eNOS signaling pathways. Apoptosis 2017; 22(5): 672-80. http://dx.doi.org/10.1007/s10495-017-1360-8 PMID: 28317089

[119] Yamagata K, Tanaka N, Matsufuji H, Chino M. β-carotene reverses the IL-1β-mediated reduction in paraoxonase-1 expression *via* induction of the CaMKKII pathway in human endothelial cells. Microvasc Res 2012; 84(3): 297-305. http://dx.doi.org/10.1016/j.mvr.2012.06.007 PMID: 22750393

[120] Jin SW, Choi CY, Hwang YP, *et al.* Betulinic Acid Increases eNOS Phosphorylation and NO Synthesis *via* the Calcium-Signaling Pathway. J Agric Food Chem 2016; 64(4): 785-91. http://dx.doi.org/10.1021/acs.jafc.5b05416 PMID: 26750873