

Natural Products and Atherosclerosis – An Overview

Francislaine Aparecida Livero¹, Lorena Neris Barboza¹, Marcia Arantes Marques², Emerson Luiz Botelho Lourenco² and Arquimedes Gasparotto Junior^{1*}

¹Laboratory of Electrophysiology and Cardiovascular Pharmacology, Faculty of Health Sciences, Federal University of Grande Dourados, MS, Brazil

²Laboratory of Pharmacology of Natural Products, University Paranaense, PR, Brazil

***Corresponding author:** Arquimedes Gasparotto, Laboratory of Electrophysiology and Cardiovascular Pharmacology, Faculty of Health Sciences, Federal University of Grande Dourados, MS, Brazil, Tel: +55 (67) 3410-2333; Fax: +55 (67) 3410-2321; Email: arquimedesgasparotto@gmail.com

Published Date: May11, 2016

ABSTRACT

According to World Health Organization Cardiovascular Diseases are listed first among the major causes of worldwide mortality. Among the main underlying causes of CDV development is atherosclerosis. Knowledge of the atherosclerosis pathogenesis of has evolved since the last century and the initial proposal that only lipids disruption was involved in disease is over passed. Actually, a set of classical and emerging risky factors like circulating lipid accumulation, production of reactive oxygen and nitrogen species, synthesis and cell proliferation, and inflammation have been correlated and are responsible for plaque formation or fatty streaks. Since atherosclerosis is a slowly and progressive disease, the therapy should be long-term, resulting in tachyphylaxis, long-term toxicity and cost, which can cause problems with adherence to conventional medications. Thus, drugs based on natural products can be a good therapeutic strategy. Although culturally different populations throughout the world utilize many natural products, only in recent years controlled clinical studies showed scientific evidence on the vasoprotective benefits of these

compounds. In general, clinical trials show that the natural products exert their effects on several important aspects involved in the atherosclerotic process, including serum lipid profile, endothelial function, inflammation, oxidative stress, and platelet aggregation/coagulation. Evidence suggests that the rational use of some natural products as an add-on therapy can preserve endothelial function and prevent the accumulation of atherosclerotic plaque that can contribute to heart attack, stroke, and other cardiovascular diseases.

Keywords: Natural products; Atherosclerosis; Medicinal plants; Dyslipidemia

GENERAL BACKGROUND

According to World Health Organization (**WHO**) Cardiovascular Diseases (**CVD**) have been listed first among the major causes of worldwide mortality for over 15 years. WHO data also indicate that in 2011, approximately 17.3 million people died worldwide as a result of CVD, most of which (80%) recorded in westernized countries. An alarming statistic indicates that this number could reach 23.6 million in 2030 if effective interventions will not have been proposed [1].

Among the main underlying causes of CVD development is atherosclerosis. The term, from Greek origin, consists in two parts: atherosis, characterized by fat accumulation accompanied by macrophages; and sclerosis, featured by fibrosis layer comprising smooth muscle cells, connective tissue and leukocyte [2]. Atherosclerosis is a multifactorial, slow and progressive disease, endpoint of a series of highly specific molecular and cellular reactions in response to endothelial aggression that results in formation of atherosclerotic plaques in the blood vessels, affecting mainly the intima of medium and large-caliber arteries [2-4].

The incidence of atherosclerosis increases exponentially in adults over 45 years and is considered a non-modifiable disorder, although some studies have found atherosclerotic plaques in young adults, suggesting that this disorder can also occur earlier [5-12]. However, actually it is established that atherosclerosis is not a simple and inevitable degenerative consequence of aging, but an inflammatory condition that can be converted into a clinical and acute event caused by the rupture of the plaque and thrombus formation [13].

THE FORMATION OF ATHEROSCLEROTIC PLAQUES

Knowledge of atherosclerosis pathogenesis has evolved since the last century and the initial proposal that only lipids disruption was involved in disease is overpassed [14]. Actually, a set of classical and emerging risk factors like circulating lipid accumulation, reactive oxygen and nitrogen species (**ROS/RNS**) production, synthesis and cell proliferation, and inflammation have been correlated and are responsible for plaque formation or fatty streaks, which usually cause obstruction [8,15-17]. Although any artery can be affected, the main disease targets are the large arteries, such as the aorta, coronary and cerebral, leading to thromboembolic clinical manifestations, including coronary artery disease, intermittent lameness of lower limbs and cerebral vascular accidents [18-20].

Three central pillars are involved in atherogenesis process: 1) fatty streaks formation, 2) atheroma formation, and 3) atherosclerotic plaques formation and atherothrombosis [21].

Fatty Streaks Formation

Animal and human studies pointed fatty streaks as the first sign of atherosclerosis [21]. Lipoproteins, especially low-density lipoprotein (**c-LDL**) plays a prominent role in the atherosclerosis etiology, though, many individuals develop cardiovascular disease in the absence of abnormalities in the lipoprotein profile [8].

Atherogenesis begins with the aggression on the arterial wall mediated by one or more risky factors, which facilitates the c-LDL penetration in the intimal tunica. In general, this process is dependent on oxidative modification that occurs in molecules of LDL-C (**ox-LDL**) due to contact with ROS/RNS, which makes them reactive and immunogenic [2,22].

The LDL-ox molecules are retained in the tunica intima by a process mediated by cytokines synthesized by vascular endothelium, including tumor necrosis factor (**TNF- α**), interleukin-1, -4 and -6 (IL-1, IL-4 and IL-6, respectively) and interferon gamma (**IFN- γ**) [23]. These cytokines promote the expression of leukocyte adhesion molecules on the endothelial surface, especially vascular cell adhesion molecules (**VCAMs**), intercellular adhesion molecules (**ICAMs**) and E-selectin. These also called acute phases of adhesion molecules are responsible for lymphocytes and monocytes attraction into the arterial wall's intima [23-25].

Through stimuli of induced chemotactic proteins, like monocyte chemotactic protein-1 (**MCP-1**), granulocyte-macrophage colony-Stimulating factor (**M-CSF**) and IL-8, monocytes migrate into the sub endothelial space and differentiate into macrophages [8]. Then, the macrophages recognize ox-LDL molecules through "scavenger" receptors and realize phagocytosis. Finally, filled with lipid inclusions, macrophages become foam cells, common characteristic of the initial atherosclerosis macroscopic lesions [8,26].

Atheroma Formation

During the maturing of atherosclerotic plaque it occurs smooth muscle cells migration of tunica media to tunica intima of arteries, which induces extracellular matrix production that form part of the fibrous atherosclerotic plaque cap. Under fibrous cap, there is a necrotic center comprising lipids and cellular debris released as a result of foam cells deaths [13,27]. The atherosclerotic plaque grows slowly and, occasionally, may completely block the artery. This process is mediated by cytokines and growth factors that are secreted by tissue macrophages, allowing the development of atheromatous lesions in their mature form [13,27].

Atherosclerotic Plaques Formation and Atherothrombosis

Macrophages present in the necrotic lesion center secrete metalloproteinases, such as collagenase and elastase which can degrade the extracellular matrix of the fibrous cap causing

rupture of the coating. With the fibrous cap rupture, the highly thrombogenic contents are exposed, initiating the coagulation process, recruiting platelets and subsequent formation of an overlying thrombus. This process, also known as atherothrombosis, is a major atherosclerosis determinant, which can lead to major acute cardiovascular events such as myocardial infarction and stroke [8,20,28] (Figure 1).

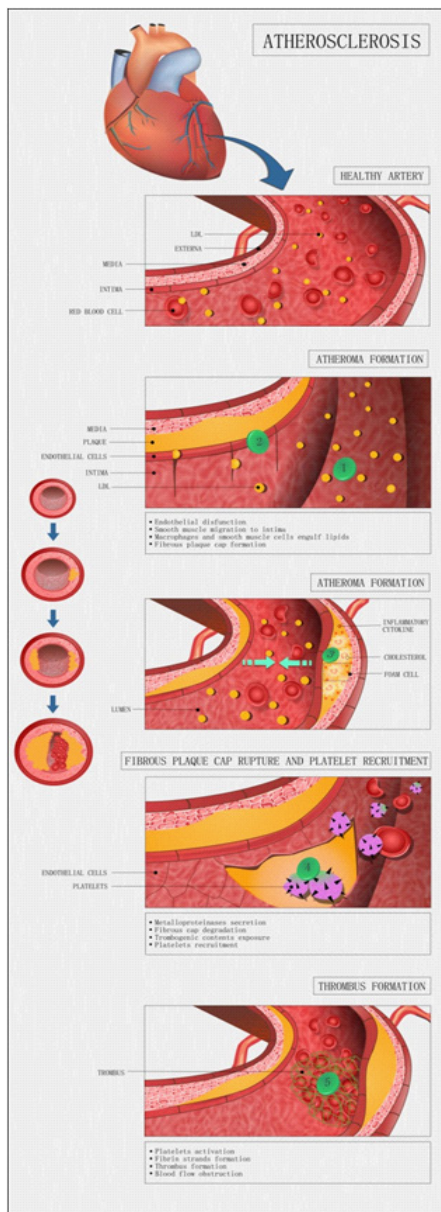


Figure 1: Pathways involved in atherothrombosis pathogenesis and targets to natural products. As a result of increasing levels of LDL and endothelial dysfunction, oxidized LDL tends to natural products.

As a result of increasing levels of LDL and endothelial dysfunction, oxidized LDL tends to penetrate on the endothelial surface and be absorbed by the endothelial cells. Macrophages recognize ox-LDL molecules and realize phagocytosis. Then, filled with lipid inclusions, macrophages become foam cells, forming a plaque between the intima and media. With plaque increase and progression, a projection to lumen occurs, resulting in reduced blood flow. Epithelial cells can rupture and activate the platelets to form fibrin strands, leading to a thrombus formation that blocks blood flow through the artery. Natural products can act in several atherothrombosis pathogenesis formation, represented by 1) anti-lipid effects; 2) enhancement of endothelial function; 3) regulation of inflammatory process; 4) platelet aggregation and 5) coagulation.

Involvement of Oxidative Stress

Oxidative stress is both a cause and a consequence of atherosclerosis and some ROS are related to vascular dysfunction through the initiation of several signaling and transcriptional pathways [29,30]. Exacerbate ROS and RNS production can lead to oxidative damage to cells, proteins and lipids, which contributes directly to endothelial dysfunction and leads to hypertension and atherosclerosis development, mainly due to oxidative modifications in LDL particles [31,32]. Macrophages and endothelial cells have several oxidizing enzymes, identified in early atherosclerotic disease, that participate in smooth muscle cells proliferation and can produce a wide range of ROS/RNS [33]. Among these enzymes are highlighted NADPH oxidase, xanthine oxidase, lipoxygenase, myeloperoxidase and nitric oxide synthase [17,34,35].

During atherosclerotic process, a strong involvement of endothelial (NOS-3 or e-NOS) and inductive (NOS-2 or iNOS) nitric oxide synthase can occur. The eNOS uncoupling, due to the absence of L-arginine or tetrahydrobiopterin (BH₄), important cofactors for NO production, causes the reduction of O₂ to O₂•⁻, leading to simultaneous changes in O₂•⁻, NO and ONOO⁻, an effective oxidant that contributes directly to endothelial dysfunction and consequently atherogenesis [36]. These redox imbalance makes blood vessels and myocardium NADPH oxidase a substantial source of O₂•⁻, promoting oxidative stress [31,34].

In a general way, biological system has a balance between ROS/RNS production and neutralization, maintained by an efficient enzymatic and non-enzymatic antioxidant system. superoxide dismutase (**SOD**), catalase (**Cat**), and glutathione peroxidase (**GPX**) are extremely important enzymes that act in an integrate and efficient H₂O₂, which can be degraded in H₂O by catalase or GPx [37,38]. The non-enzymatic antioxidant system is constituted by way to reduce oxidative compounds and prevent lipoperoxidation. To exemplify, SOD promotes O₂•⁻ dismutase into a great variety of substances such as glutathione, vitamins, minerals and various polyphenolic compounds, that may be endogenous or obtained from diet [37,38].

ANTIATHEROGENIC EFFECTS OF NATURAL PRODUCTS

Since atherosclerosis is a slowly and progressive disease, the therapy should be long-term, resulting in tachyphylaxis, long-term toxicity and cost, which can cause problems with adherence to conventional medications. Thus, drugs based on natural products can be a good therapeutic strategy [39]. Several natural products have been tested in different animal models, and many of them have shown to be effective in atherosclerosis prevention and treatment. Moreover, studies in human patients are more restricted, but they demonstrate an important role of these agents in the prevention and treatment of this pathology. In general, clinical trials show that the available natural products exert their effects on five important aspects involved in the atherosclerotic process: 1) serum lipid profile, 2) endothelial function, 3) inflammation, 4) oxidative stress, and 5) platelet aggregation/coagulation.

Anti-Lipid Effects

Several clinical studies have been performed to assess the anti-atherosclerotic effects of natural products, especially on lipid disruption. Through HMG-CoA reductase and cholesterol synthesis blocking, *Allium sativum* L., resveratrol and *Nigella sativa* L. increases c-HDL, reduces serum levels of triglycerides, cholesterol and c-LDL, as well as c-LDL oxidation [40-43]. Beyond that, Ko and collaborators [44] explored the clinical effects of ginseng (*Panax ginseng* C.A. Meyer) on serum lipid profile and founded decreased levels of triglycerides, cholesterol and c-LDL, besides c-HDL augment in patients with hypercholesterolemia, through lipid accumulation suppressing and increase of adiponectin expression. However, lipid-lowering mechanism of some natural products like *Cynara scolymus* L. are not totally elucidated, but it seems to involve regulation of lipid catabolism genes [45].

Enhancement of Endothelial Function

Endothelial dysfunction is due to a number of changes in the mechanisms that regulate the dilation of blood vessels caused by unbalanced diet, sedentary lifestyle, smoking, diabetes, high blood pressure and high cholesterol levels. It is now known that endothelial dysfunction is a key step in the development of atherosclerotic lesions. Several clinical studies suggest that many natural products can reduce the formation and evolution of atherosclerotic lesions through improved endothelial function. In fact, previous studies reported the vasodilator and circulation beneficial effects of *Ginkgo biloba* L., through eNOS expression modulation [46]. *Allium sativum* L. also improve endothelial function, inhibit endothelial cell damage, and transform smooth muscle cells [47-49]. On the other hand, many studies have shown that polyphenols from several natural products also ameliorate endothelial dysfunction through increasing serum levels of nitrate and NO [50,51].

Regulation of Inflammatory Processes

Currently, it is well established that atherosclerosis is not a simple and inevitable consequence of degenerative aging, but an inflammatory condition that can be reduced through the action of many secondary metabolites present in various natural products. In fact, in the last decade, we observed consistent data on the anti-inflammatory properties of natural products during the development of atherosclerosis in humans. As an example, *Terminalia arjuna* Wight and Arn., an important medicinal specie cited by Ayurveda as a treatment for heart disease since the 7th century CE, promotes anti-inflammatory effects in patients with coronary artery disease through suppression of TNF α , VCAM and ICAM [52]. In the same way, the beneficial modulating effects of *Allium sativa* L. in atherosclerotic patients were confirmed in the *in vitro* study by Rassou et al. [53] that related antiinflammatory effects through inhibition of VCAM and ICAM and consequent reduction of leukocyte migration to vascular endothelium. Similarly, *Curcuma longa* L. has also presented antiinflammatory effects on cardiovascular diseases, in a NF- κ B inhibition mechanism [54].

Antioxidant Function

For many years it is known that natural products, especially those rich in polyphenol compounds show significant antioxidant properties. As for the genesis of atherosclerotic disease, cholesterol oxidation is a limiting step, it is to be expected that different metabolites present in natural products can play significant vasoprotective effects. The main natural products antioxidant mechanisms of action are through ROS concentration decreasing or production blocking and lipid chain oxidation inhibiting [21]. Antioxidant protection is linked to reduction of lipoproteins oxidative change and lipid peroxidation prevention, since it is considered an atherosclerosis key event [21]. The most antioxidants effects of natural products are attributed to phenolic compounds and their moderate consumption have additional benefits in the atherosclerosis, prevention and development decreasing blood pressure and reducing platelet aggregation [21,51,55]. Ramirez-Tortosa et al. [56] and Khoo et al. [42] found that extra-virgin olive oil and *Allium sativum* L. increases the LDLresistance to oxidation. Similarly, by reducing the lipid peroxidation and superoxide anion production, a mixture obtained from *Panax ginseng* C.A. Meyer and *Crataegus monogyna* Jacq. (Lindm.) was able to significantly reduce blood lipids in human patients [44]. In fact, this cannot be considered an isolated event. In a double blind controlled clinical trial *Zingiber officinale* Roscoe was also able to cause lipid-lowering effects dependently of their antioxidant activity [57]. On the other hand, although experimental studies indicate a promising effect of the vitamin E (a classic antioxidant) in reducing atherosclerosis, clinical trials are controversial [58-60].

Platelet Aggregation/Coagulation

No less importantly, the protective effects on the late atherosclerosis stage, i.e. the prevention of atherothrombosis, became the focus of much research involving natural products. In fact, it

is now known that through suppression of NADPH-oxidase and oxidative inactivation of SH2 domain-containing protein tyrosine phosphatase-2, resveratrol inhibits collagen-induced platelet stimulation [61]. In the same way, the effects of *Allium sativum* L. under activity fibrinolytic, platelet aggregation and thrombin formation were described by Bordia et al. [62]. Finally, besides its well recognized antioxidant effects, polyphenols can act synergistically in inhibiting platelet recruitment by a NO and O₂[•] dependent mechanism [63].

CONCLUDING REMARKS

In recent years several clinical evidences describe the benefit of natural products in preventing and reducing the atherosclerosis development. Although culturally different populations throughout the world use a lot of natural product, only in recent years controlled clinical studies showed scientific evidence on the vasoprotective benefits of these compounds. If we look at a horizon not so far, we see that the rational use of some natural products and their phyto-derived, can become important add-on therapy options to conventional drugs, adding to the efficacy and safety of these compounds, an important ethno-cultural appeal transmitted from generation to generation.

References

- Mendis S, Puska P, Norrving B. World Health Organization (WHO) Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: World Health Organization. 2011.
- Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999; 340: 115-126.
- Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993; 362: 801-809.
- Wang T, Palucci D, Law K, Yanagawa B, Yam J, et al. Atherosclerosis: pathogenesis and pathology. *Cardiovascular Pathology*. 2012; 1: 461-467.
- Buckley ML, Ramji DP. The influence of dysfunctional signaling and lipid homeostasis in mediating the inflammatory responses during atherosclerosis. *Biochimica et Biophysica Acta*. 2015; 1052: 1498-1510.
- Hazzard WR. Atherosclerosis and aging: a scenario in flux. *Am J Cardiol*. 1989; 63: 20-24.
- Liu HH, Li JJ. Aging and dyslipidemia: a review of potential mechanisms. *Ageing Res Rev*. 2015; 19: 43-52.
- Libby P. Inflammation in atherosclerosis. *Nature*. 2002; 420: 868-874.
- McGill HC, McMahan CA, Zieske AW, Tracy RE, Malcom GT, et al. Association of Coronary Heart Disease Risk Factors with microscopic qualities of coronary atherosclerosis in youth. *Circulation*. 2000; 102: 374-379.
- McLaren JE, Michael JE, Ashlin TG, Ramji DP. Cytokines, macrophage lipid metabolism and foam cells: Implications for cardiovascular disease therapy. *Progress in Lipid Research*. 2011; 50: 331-347.
- Satilmis S, Celik O, Biyik I, Ozturk D, Celik K, et al. Association between serum vitamin D levels and subclinical coronary atherosclerosis and plaque burden/composition in young adult population. *Bosnian Journal of Basic Medical Sciences*. 2015; 8: 67-72.
- Terra LN, Ehlers OR, Clemente E, Jeckel-Neto EA. Ácidos graxos ômega 3 e aterosclerose. *Aspectos Biológicos e Geriátricos do Envelhecimento*. Porto Alegre: EDIPUCRS. 1998; 135-147.
- Lusis AJ. Atherosclerosis. *Nature*. 2000; 407: 233-241.
- Ross R, Harker L. Hyperlipidemia and atherosclerosis. *Science*. 1976; 193: 1094-1100.
- Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). *Arteriosclerosis, Thrombosis and Vascular Biology*. 2002; 22: 1162-1167.
- Gottlieb GVM, Bonardi G, Moriguchi EH. Physiopathology and inflammatory aspects of atherosclerosis. *Scientia Medica*. 2005; 15: 203-207.

17. Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis*. 2014; 237: 208-219.
18. Aboyans V, Lacroix P, Criqui MH. Large and small vessels atherosclerosis: similarities and differences. *Prog Cardiovasc Dis*. 2007; 50: 112-125.
19. Badimon JJ, Fuster V, Chesebro JH, Badimon L. Coronary atherosclerosis. A multifactorial disease. *Circulation*. 1993; 87: 113-16.
20. Ouweneel AB, Van Eck M. Lipoproteins as modulators of atherothrombosis: From endothelial function to primary and secondary coagulation. *Vascul Pharmacol*. 2015.
21. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. *Int J Prev Med*. 2014; 5: 927-946.
22. Kishimoto Y, Yoshida H, Kondo K. Potential Anti-Atherosclerotic Properties of Astaxanthin. *Mar Drugs*. 2016; 14.
23. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res*. 2001; 89: 763-771.
24. Kirichenko TV, Sobenin IA, Nikolic D, Rizzo M, Orekhov AN. Anti-cytokine therapy for prevention of atherosclerosis. *Phytomedicine*. 2015.
25. Nikoiforov NG, Gratchev AN, Sobenin IA, Orekhov AN, Kzhyhskowska YG. [Interaction of native and modified low density lipoprotein with intimal cells in atherosclerotic lesion]. *Patol Fiziol Eksp Ter*. 2013; 109-117.
26. Lind L. Circulating markers of inflammation and atherosclerosis. *Atherosclerosis*. 2003; 169: 203-214.
27. Patel S, Celermajer DS, Bao S. Atherosclerosis-underlying inflammatory mechanisms and clinical implications. *Int J Biochem Cell Biol*. 2008; 40: 576-580.
28. Schonbeck U, Mach F, Sukhova GK, Herman M, Graber P, et al. CD40 ligation induces tissue factor expression in human vascular smooth muscle cells. *The American Journal of pathology*. 2000; 156: 7-14.
29. Kim YW, Byzova TV. Oxidative stress in angiogenesis and vascular disease. *Blood*. 2014; 123: 625-631.
30. Matthews AT, Ross MK. Oxymetazoline, Endocannabinoids, and Atherosclerosis. *Toxics*. 2015; 3: 481-498.
31. Vara D, Pula G. Reactive oxygen species: physiological roles in the regulation of vascular cells. *Curr Mol Med*. 2014; 14: 1103-1125.
32. Vaziri ND, Wang XQ, Oveis F, Rad B. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension*. 2000; 36: 142-146.
33. Touyz RM. Molecular and cellular mechanisms in vascular injury in hypertension: role of angiotensin II. *Curr Opin Nephrol Hypertens*. 2005; 14: 125-131.
34. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation*. 2003; 108: 1912-1916.
35. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, et al. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. *Circulation Research*. 2000; 86: 85-90.
36. Kawashima S, Yokoyama M. Dysfunction of endothelial nitric oxide synthase and atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2004; 24: 998-1005.
37. Lubrano V, Balzan S. Enzymatic antioxidant system in vascular inflammation and coronary artery disease. *World J Exp Med*. 2015; 5: 218-224.
38. Orekhov AN, Tertov VV, Kudryashov SA, Smirnov VN. Trigger like stimulation of cholesterol accumulation and DNA and extracellular matrix synthesis induced by atherogenic serum or low density lipoprotein in cultured cells. *Circ Res*. 1990; 66: 311-320.
39. Orekhov AN, Sobenin IA, Korneev NV, Kirichenko TV, Myasoedova VA, Melnichenko AA, Balcells M. Anti-atherosclerotic therapy based on botanicals. *Recent Pat Cardiovasc Drug Discov*. 2013; 8: 56-66.
40. Ahmad Alobaidi AH. Effect of *Nigella sativa* and *Allium sativum* coadministered with simvastatin in dyslipidemia patients: a prospective, randomized, double-blind trial. *Anti-inflammatory & Anti-allergy Agents in Medicinal Chemistry*. 2014; 13: 68-74.
41. Jain AK, Vargas R, Gotzkowsky S, McMahon FG. Can garlic reduce levels of serum lipids? A controlled clinical study. *Am J Med*. 1993; 94: 632-635.
42. Khoo YS, Aziz Z. Garlic supplementation and serum cholesterol: a meta-analysis. *J Clin Pharm Ther*. 2009; 34: 133-145.
43. Tome-Carneiro J, Gonzalez M, Larrosa M, Garcia-Almagro FJ, Aviles-Plaza F, et al. Consumption of a grape extract supplement containing resveratrol decreases oxidized LDL and ApoB in patients undergoing primary prevention of cardiovascular disease: a triple-blind, 6-month follow-up, placebo-controlled, randomized trial. *Molecular Nutrition & Food Research*. 2012; 56: 810-821.

44. Ko CN, Park SU, Chang GT, Jung WS, Moon SK, Jung-Mi Park, et al. Antihyperlipidemic and antioxidant effects of the mixture of *Ginseng radix* and *Crataegi fructus*: experimental study and preliminary clinical results. *Journal of Ginseng Research*. 2011; 35: 162-169.
45. Bundy R, Walker AF, Middleton RW, Wallis C, Simpson ACR Artichoke leaf extract (*Cynarascolymus*) reduces plasma cholesterol in otherwise healthy hypercholesterolemic adults: A randomized, double blind placebo controlled Trial. *Phytomedicine*. 2008; 15: 668-675.
46. Wu YZ, Li SQ, Zu XG, Du J, Wang FF Ginkgo biloba extract improves coronary artery circulation in patients with coronary artery disease: contribution of plasma nitric oxide and endothelin-1. *Phytotherapy Research*. 2008; 22: 734-739.
47. Ho SE, Ide N, Lau BH. S-allyl cysteine reduces oxidant load in cells involved in the atherogenic process. *Phytomedicine*. 2001; 8: 39-46.
48. Pittler MH1, Ernst E. Complementary therapies for peripheral arterial disease: systematic review. See comment in PubMed Commons below Atherosclerosis. 2005; 181: 1-7.
49. Williams MJ, Sutherland WH, McCormick MP, Yeoman DJ, de Jong SA. Aged garlic extract improves endothelial function in men with coronary artery disease. *Phytother Res*. 2005; 19: 314-319.
50. Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, et al. Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. *Journal of the American College of Cardiology*. 2005; 46: 1864-1868.
51. Stoclet JC, Chataigneau T, Ndiaye M, Oak MH, El Bedoui J, Chataigneau M, Schini-Kerth VB. Vascular protection by dietary polyphenols. *Eur J Pharmacol*. 2004; 500: 299-313.
52. Kapoor D, Trikha D, Dhawan V, Vijayvergiya R. Modulation of inflammatory genes by *Terminalia arjuna* in coronary artery disease. In: *Proceedings of 11th Annual Conference of International Society for Heart Research (Indian Section)*. 2014; 9: 46-47.
53. Rassoul F, Salvetter J, Reissig D, Schneider W, Thiery J, et al. The influence of garlic (*Allium sativum*) extract on interleukin 1 α -induced expression of endothelial intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. *Phytomedicine*. 2006; 13: 230-235.
54. Shaygani E, Bahmani M, Asgary S, Rafieian-Kopaei M. Inflammaging and cardiovascular disease: Management by medicinal plants. *Phytomedicine*. 2015.
55. Quiñones M, Miguel M, Aleixandre A. Beneficial effects of polyphenols on cardiovascular disease. *Pharmacol Res*. 2013; 68: 125-131.
56. Ramirez-Tortosa MC, Urbano G, López-Jurado M, Nestares T, Gomez MC, et al. Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. *J Nutr*. 1999; 129: 2177-2183.
57. Alizadeh-Navaei R, Roozbeh F, Saravi M, Pouramir M, Jalali F, Moghadamnia AA. Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. *Saudi Medical Journal*. 2008; 29: 1280-1284.
58. Boaz M, Smetana S, Weinstein T, Matas Z, Gafer U, et al. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. 2000; 356: 1213-1218.
59. Ozkanlar S, Akcay F. Antioxidant vitamins in atherosclerosis--animal experiments and clinical studies. *Adv Clin Exp Med*. 2012; 21: 115-123.
60. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *The New England Journal of Medicine*. 2000; 342: 154-160.
61. Jang JY, Hyun Min J, Wang SB, Clae WY, Baek JW, et al. Resveratrol inhibits collagen-induced platelet stimulation through suppressing NADPH oxidase and oxidative inactivation of SH2 domain-containing protein tyrosine phosphatase-2. *Free Radical Biology and Medicine*. 2015; 89: 842-851.
62. Bordia A, Verma SK, Srivastava KC Effect of garlic (*Allium sativum*) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 1998; 58: 257-263.
63. Pignatelli P, Di Santo S, Buchetti B, Sanguigni V, Brunelli A, Violi F. Polyphenols enhance platelet nitric oxide by inhibiting protein kinase C-dependent NADPH oxidase activation: effect on platelet recruitment. 2006; *FASEB Journal* 20: 1082-1089.