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REVIEW

Diosgenin and Its Analogs: Potential Protective Agents Against Atherosclerosis

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Abstract: Atherosclerosis is a chronic inflammatory disease of the artery wall associated with lipid metabolism imbalance and maladaptive immune response, which mediates most cardiovascular events. First-line drugs such as statins and antiplatelet drug aspirin have shown good effects against atherosclerosis but may lead to certain side effects. Thus, the development of new, safer, and less toxic agents for atherosclerosis is urgently needed. Diosgenin and its analogs have gained importance for their efficacy against life-threatening diseases, including cardiovascular, endocrine, nervous system diseases, and cancer. Diosgenin and its analogs are widely found in the rhizomes of *Dioscore, Solanum*, and other species and share similar chemical structures and pharmacological effects. Recent data suggested diosgenin plays an anti-atherosclerosis role through its anti-inflammatory, antioxidant, plasma cholesterol-lowering, anti-proliferation, and anti-thrombotic effects. However, a review of the effects of diosgenin and its natural structure analogs on AS is still lacking. This review summarizes the effects of diosgenin and its analogs on vascular endothelial dysfunction, vascular smooth muscle cell (VSMC) proliferation, migration and calcification, lipid metabolism, and inflammation, and provides a new overview of its anti-atherosclerosis mechanism. Besides, the structures, sources, safety, pharmacokinetic characteristics, and biological availability are introduced to reveal the limitations and challenges of current studies, hoping to provide a theoretical basis for the clinical application of diosgenin and its analogs and provide a new idea for developing new agents for atherosclerosis.

Keywords: atherosclerosis, diosgenin, dioscin, analogs

Introduction

Cardiovascular diseases are the leading cause of death worldwide. According to the World Health Organization, about 17.9 million people die from cardiovascular disease each year,[1] among which atherosclerosis is responsible for the majority of cardiovascular events.

Atherosclerosis is a chronic inflammatory disease of the artery wall. It usually appears in the subcutaneous space of medium to large arteries and is associated with lipid metabolism imbalance and maladaptive immune responses.[2] In the early stage of the disease, various pathogenic factors lead to damage of the intima, which further increases the infiltration of inflammatory cells and lipids, and accelerates the formation of foam cells. In the later stage, plaque calcification and rupture occur, leading to the formation of thrombosis and, in turn, ischemic injury syndromes of vital organs, such as myocardial infarction, unstable angina (ischemic heartache), stroke, and other complications.[3] Lowering blood lipids, controlling blood pressure, dilating blood vessels, preventing platelet aggregation from preventing thrombotic complications, etc., are some of the treatment methods for atherosclerosis. Although these therapies alleviate the occurrence and progression of atherosclerosis, they do not target the inflammatory mechanisms responsible for the progression of atherosclerosis. Also, first-line drugs such as statins and antiplatelet drug aspirin in the secondary prevention of ACS have been associated with some adverse reactions such as myitis and myalgia (for statins),[4] gastrointestinal bleeding, ulcers, and increased drug resistance (for antiplatelet drug aspirin).[5,6] Thus, the development of new, safer, and less toxic agents for atherosclerosis is urgently required.

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At present, phytochemicals have been attracting increasing attention due to their low toxicity and high yield. Diosgenin and its analogs are important natural steroidal saponins used as active ingredients of dioscin tablets, Di'ao Xin Xue Kang capsules, Dunye Guan Xin Ning, and other medicines which have been used in China for more than 20 years to treat coronary heart disease and other cardiovascular diseases.[7–9] Many experimental studies and some clinical trials have demonstrated that diosgenin and its analogs have anti-inflammatory, antioxidant, plasma cholesterol-lowering, anti-proliferation, and anti-thrombotic effects, thus suggesting that these drugs may be promising candidates for atherosclerosis treatment.[10,11] However, there is still a lack of relevant summaries.

In this review, we summarized the effects of diosgenin and its analogs on vascular endothelial dysfunction, vascular smooth muscle cell proliferation, migration and calcification, lipid metabolism, and inflammation, thus providing a new overview of its anti-atherosclerosis mechanism. Besides, the structures, sources, safety, pharmacokinetic characteristics, and biological availability were introduced, hoping to provide a new idea for the development of new agents for atherosclerosis.

Overview of the Structures, Sources, and Safety of Diosgenin and Its Analogs

Diosgenin is a natural steroidal saponin, whose structure is very similar to endogenous steroids (such as cholesterol, progesterone, and estrogen). Due to its estrogenic activity, it is often used as a precursor for producing norethindrone and progesterone and as a dietary supplement in hormone replacement therapy to improve menopausal symptoms.[12] Diosgenin has many structural analogs. Dioscin is the glycoside form of diosgenin obtained by connecting trisaccharide alpha-L-Rha-(1->4)-[alpha-L-Rha-(1->2)]-beta-D-Glc to diosgenin at position 3 through a glycosidic bond;[13] it can be converted to diosgenin by hydrolysis.[14] Considering that chemical structures and effects on lipid bilayer membranes of dioscin and diosgenin are similar to those of cholesterol, they have an essential role in cholesterol metabolism, ie, inhibition of the intestinal absorption of dietary cholesterol and the acceleration of the transformation of cholesterol into bile acids.[15–17]

Methylprotodioscin (MPD), pseudoprotodioscin (PPD), protodioscin (PD), yamogenin and tomatidine are other analogs of diosgenin that have similar pharmacological effects and are mainly extracted from the roots and stems of Trigonella, Smilax, Dioscorea, Solanum, and Costus species, among which Dioscorea has the highest content.[18–20] The molecular formula, molecular weight, source, and chemical structure of diosgenin and its main analogs are shown in Table 1 and Figure 1.

Safety is often the first step in drug development. Natural steroid saponins usually have high safety. Preclinical studies showed mild subchronic toxicity in male rats but not in female rats treated with diosgenin. Daily administration of diosgenin above 300 mg/kg may cause mild gastrointestinal distension, hemolytic anemia, and weight loss in rats, while long-term use of steroidal saponins in large doses has been reported to damage the liver, leading to liver damage such as acute icteric hepatitis.[21] However, at a moderate dose, diosgenin showed a significant protective effect on liver injury induced by ethanol and paracetamol.[22] Tohda et al suggested that the oral toxicity dosage (LD50) of diosgenin to mice and rats is > 8000 mg/kg (> 480g/ human).[23] Moreover, diosgenin derivative compound 5 did not show any toxicity in mice at an oral dose of 575.5 mg/ kg.[24] Therefore, diosgenin and its analogs are considered safe and non-toxic at the conventional dosage;[25] however, the safety of other analogs needs to be further explored.

Material and Methods Regulation of Endothelial Dysfunction

Endothelial cells are an important barrier between the vascular wall and blood. When exposed to risk factors such as excess lipid (LDL), hypertension (shear stress), oxygen-free radicals, cigarette smoke constituents, high blood sugar, and stress, endothelial cell structure and function may change, which may result in endothelial dysfunction, which, in turn, can induce atherosclerosis. In the early stage of atherosclerosis, endothelial-dependent vasodilation is impaired, oxidative stress is enhanced, and leukocytes increase with the help of adhesion molecules. In the late stage, plaque rupture and thrombosis are formed.[26–30]

Table 1 Molecular Formula, Relative Molecular Weight and Main Sources of Diosgenin and Its Analogues

Compound	Molecular Formula	Relative Molecular Weight (g/mol)	Main Source	References
Diosgenin	$C_{27}H_{42}O_3$	414.6	The roots of Dioscorea villosa, the seeds of fenugreek (T. foenum graecum Linn), the rhizomes of D. zingiberensis	[163,164]
Dioscin	$C_{45}H_{72}O_{16}$	869.05	The roots of Dioscorea villosa, the rhizomes of D. zingiberensis and Dioscorea nipponica	[165–168]
Pseudoprotodioscin.	$C_{51}H_{82}O_{21}$	1031.2	The seeds of fenugreek (T. foenum graecum Linn), the rhizomes of Dioscorea panthaica	[169,170]
Protodioscin	$C_{51}H_{84}O_{22}$	1049.2	The Rhizome of Dioscorea tokoro, the rhizomes of Dioscorea nipponica, the seeds of fenugreek (T. foenum graecum Linn), the seeds of Tribulus Terrestris	[171–174]
Methylprotodioscin	$C_{52}H_{86}O_{22}$	1063.2	The rhizomes of Dioscorea collettii var. hypoglauca (Dioscoreaceae)	[175]
Yamogenin	$C_{27}H_{42}O_3$	414.6	The dried stems of Asparagus officinalis L, the seeds of fenugreek (T. foenum graecum Linn)	[101,176,177]
Tomatidine	$C_{27}H_{45}NO_2$	415.7	The unripe fruits, leaves, stems and roots of tomato plant	[178]

Abbreviations: MPD, Methylprotodioscin; PPD, pseudoprotodioscin; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; eNOS, endothelial NO synthase; iNOS, inducible NO synthase; ET-1, endothelin-1; Arg-1, arginase-1; MDA, malondialdehyde; HUVECs, human umbilical vein endothelial cells; PAT, perivascular adipose tissue; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; GPX, glutathione peroxidase; Sirt3, sirtuin 3; Nrf2, nuclear factor erythroid 2-related factor 2; GR, glutathione reductase; GST, glutathione S-transferase; oxLDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; M-CSF, monocyte colony-stimulating factor; NF-κB, nuclear factor -κB; TNFR1, tumor necrosis factor receptor 1; VSMCs, vascular smooth muscle cells; PA, plasminogen activator; vwF, von Willebrand factor; Par, protease-activated receptor; TXA, tranexamic acid; TF, tissue factor; PAI-1, plasminogen activator inhibitor-1; PT, prolong prothrombin time; TT, thrombin time; APTT, activate partial thromboplastin time; FOXM1, Forkhead box protein M1; ADAM15, adamalysin metalloproteinase 15; Runx2, runt-related transcription factor 2; FC, free cholesterol; CE, cholesterol ester; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL, high-density lipoprotein; VLDL-C, very-low-density lipoprotein cholesterol; PPARγ, peroxisome proliferation-activated receptorγ; LCAT, cholesterol acyltransferase; PL, pancreatic lipase; HL, hepatic proteinase; PGC1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; LPL, lipoprotein lipase; Erα, estrogen receptor α; SREBPs, sterol response element-binding proteins; LXRs, liver X receptors; PCSK9, proprotein convertase subtilisin-like/kexin type 9; LDLR, low-density lipoprotein receptor; ACAT, Acyl-Coenzyme A: Cholesterol Acyltransferase; SRs, Scavenger receptors; RCT, reverse cholesterol transport; NPC1L1, Niemann-Pick C1-Like 1; ABCG5/8, ATP-binding cassette G5/8; SRB1, scavenger receptor class B type I; CES-1, carboxylesterase-1; CYP7A1, cholesterol7alpha- hydroxylase; FXR, farnesoid X receptor; NICD, Notch intracellular domain; MDC, macrophage-derived chemokine, BLC, B lymphocyte chemokine; MIP-1α, macrophage inflammatory protein-1alpha; LPS, lipopolysaccharide; Pam3CSK4, palmitoyl-3-cysteine-serine-lysine-4; TF, tissue factor; CYP2E1, Cytochrome P450 2E1; COX-2, cyclooxygenase-2; HMGB1, high mobility group box-1; β-CD, β-cyclodextrin; SD rats, Sprague–Dawley rats; FeCl3, the ferric chloride; ADP, adenosine diphosphate; acLDL, Acetylated-low density lipoprotein; HAECs, human aortic endothelial cells; PBMCs, peripheral blood mononuclear cells; OxyLDL, Oxidatively modified LDL; MPMs, Mouse peritoneal macrophages; LPS, Lipopolysaccharide; HUVECs, Human umbilical vein endothelial cells; HMDMs, Human monocyte-derived macrophages; CE, Cholesterol ester; EL, endothelial lipase; FC, fatty acid; GSH, Glutathione; GSSG, oxidized glutathione; E17G, estradiol-17beta-(beta-D-glucuronide); Nrf2, nuclear factor E2-related factor-2; HO-1, heme oxygenase-1; SOD, superoxide dismutase; AMPK, AMP-activated protein kinase; GSS, glutathione synthetase; JNK, junNH2- terminal kinase; sirt1, sirtuin 1.

Regulation of Vascular Tone

At the early stage, atherosclerosis is often accompanied by the reduced secretion and lower activity of NO, which leads to abnormal vasoconstriction and spasm, changes in blood flow shear stress, thrombosis, and even vascular proliferation. Therefore, NO is extremely important in the development of atherosclerosis.

As an important endothelium-derived relaxation factor, NO can dilate blood vessels through the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) signaling pathway.[31] Endothelial cells catalyze the production of NO through endothelial NO synthase (eNOS),[32] while inducible NO synthase (iNOS) mediates endothelium-dependent vasodilatation dysfunction.[33] When exposed to free fatty acids and hypercholesterolemia, superoxide anion is overexpressed in endothelial cells, and NO activity is reduced, thus attenuating NO-induced relaxation and causing endothelial dysfunction.[34,35] Endothelin-1 (ET-1) is an endothelial cell-derived peptide that inhibits eNOS activity and promotes vasoconstriction. ET-1 is increased in atherosclerotic lesions.[36]

Diosgenin can improve vascular resistance and regulate arterial tension, and this beneficial effect may be mediated by the increasing NO production and activation of eNOS.[37,38] Studies have discovered that diosgenin improves endothelial

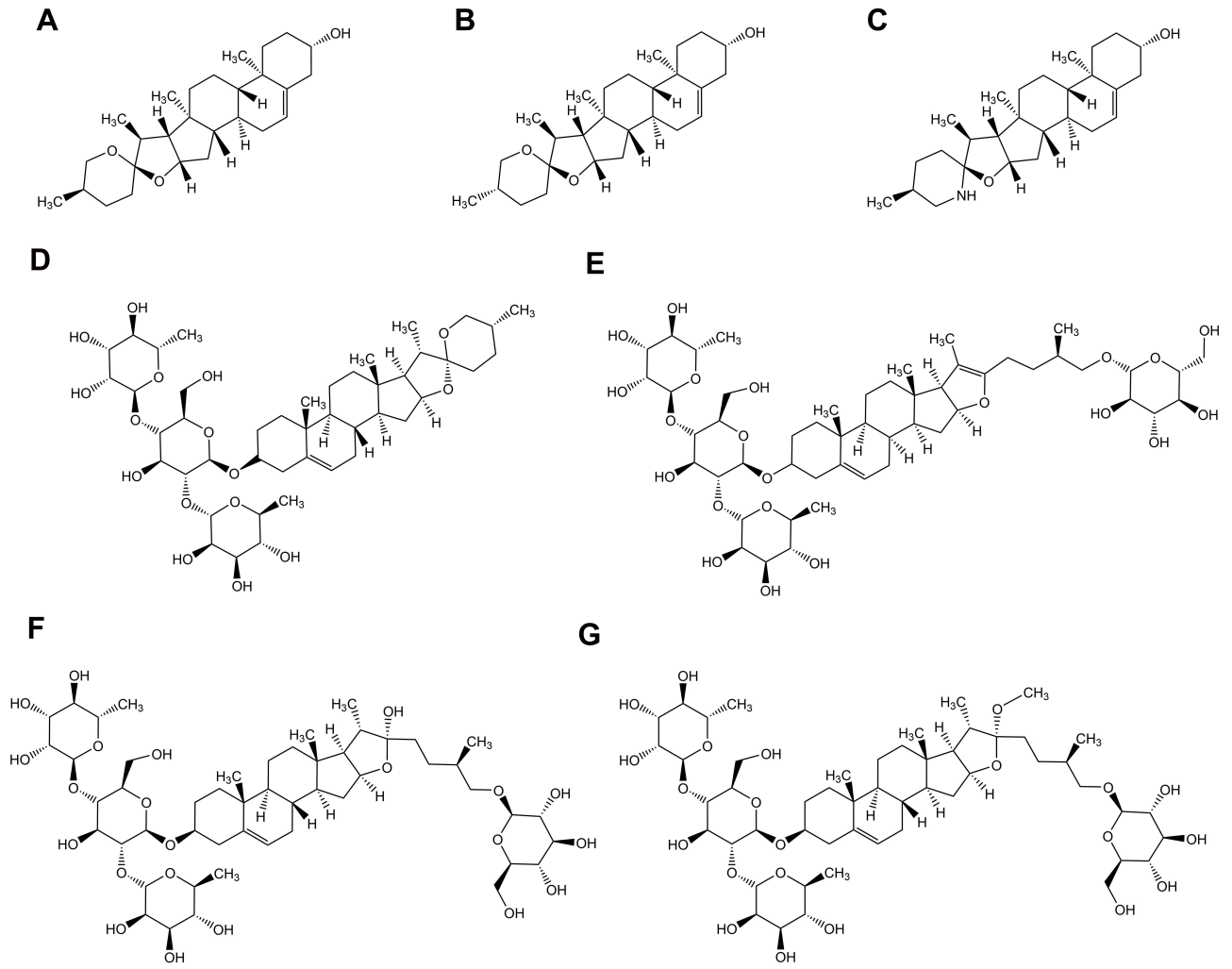

Figure 1 Structure of diosgenin and Its analogs. (**A**) diosgenin;(**B**) yamogenin;(**C**) tomatidine;(**D**) dioscin (**E**) Pseudoprotodioscin;(**F**) Protodioscin (**G**) Methylprotodioscin.

dysfunction by reducing the increase of MCP-1 and iNOS induced by atherogenic diet, promoting the expression of arginase-1 (Arg-1), inhibiting eNOS phosphorylation, and increasing NO production.[39,40] Diosgenin also restores insulin-mediated NO production and inhibits ET-1 expression via activation of the Akt pathway.[41] Furthermore, in vitro experiments suggested that PPD can regulate the levels of eNOS, NO, and malondialdehyde (MDA) in human umbilical vein endothelial cells (HUVECs), and reduce the oxidative stress of endothelial cells, which is mediated by the ERα pathway.[42] Another study suggested that perivascular adipose tissue (PAT) can regulate vascular endothelial function through endocrine or paracrine functions.[43] Also, adiponectin secreted by PAT can promote NO production in endothelial cells and dilate blood vessels.[44,45] Moreover, diosgenin can alleviate the impairment of endothelial-dependent vasodilation and improve endothelial dysfunction via affection of endothelial cells by modulating the paracrine function of PAT.[40] Thus, it is believed that diosgenin and its analogs control vascular tension by regulating enzymes related to NO metabolism and have a positive role in preventing AS at an early stage.

Regulation of Oxidative Stress

Vascular endothelial cells are highly sensitive to oxidative stress. Endothelial function is impaired when the production of antioxidants such as reactive oxygen species (ROS) exceeds the endogenous antioxidant capacity. Superoxide dismutase (SOD) is an antioxidant protein that helps remove superoxide;[46] catalase (CAT) can directly decompose H_2O_2;[47] glutathione (GSH) and glutathione peroxidase (GPX) c hyperlipidemia and pig models of coronary heart disease,

which leads an maintain the reductive state of the cell environment, remove hydrogen peroxide and lipid peroxides, and mitigate the toxicity of toxic metal ions and the damage of toxic oxygen products to cells.[48] Preclinical studies have shown that diosgenin can inhibit the generation of oxidation product MDA and oxygen free radicals by activating antioxidant enzymes (such as SOD, CAT, GPX, and eNOS) in plasma and liver of rats with to the improvement of lipid peroxidation and combats oxidative stress in the aorta.[49–55]

Sirtuin 3 (Sirt3) is closely associated with oxidative stress, lipid metabolism, and inflammation and is conducive to superoxide clearance.[56] The Nuclear factor erythroid 2-related factor 2 (Nrf2) is used as the main transcription regulator of GPX, GST, SOD, CAT, and GR.[57] Dioscin can enhance antioxidant capacity and reduce oxidative stress by up-regulating Sirt3, thereby promoting the expression of antioxidant enzymes Nrf2, SOD2, and GST, and inhibiting the expression of Keap1.[58] Also, diosgenin preconditioning can reduce the production of ROS and GSH in H_2O_2-induced HUVECs cells, which have an important role in protecting endothelial integrity.[49]

To sum up, dioscin and diosgenin are good antioxidants that can inhibit oxidative stress, protect endothelium and enhance vascular function by enhancing the antioxidant system composed of SOD, GSH, CAT, GST, GR and GPX.

Inhibition of Leukocyte Adhesion

When the vascular endothelium is activated by pro-inflammatory cell signaling pathways and stimulated by oxidized lipids and low-density lipoprotein (oxLDL), endothelial cells can secrete a variety of adhesion molecules and chemokines, promoting leukocytes (monocytes and T lymphocytes) that adhere to the atherosclerotic sites. These cells then migrate and differentiate into macrophages,[59] which have an important role in initiating atherosclerosis and promoting plaque instability. In addition, endothelial cells can also secrete monocyte chemoattractant protein-1 (MCP-1) and monocyte colony-stimulating factor (M-CSF) under the stimulation of oxLDL, which further induces the recruitment of monocytes. In vitro studies have shown that dioscin and diosgenin can inhibit the expression of ICAM-1 and VCAM-1 in macrophages and HUVECs induced by TNF-α by blocking the activation of nuclear factor -κB (NF-κB), thus reducing the adhesion of monocytes to HUVEC stimulated by TNF-α.[60] Moreover, in vivo studies found that diosgenin can reduce the expression of MCP-1 in the aorta of atherosclerotic rats, reduce inflammation,[61] and reduce TNF-α reactivity by inducing the shedding of the extracellular domain of tumor necrosis factor receptor 1 (TNFR1). This, in turn, reduces the expression of ICAM-1 induced by TNF-α and the inflammatory response of vascular endothelial cells.[62] At the same time, PPD can reduce the inflammatory response of the arterial wall of HUVECs and ovariectomized apoE-/- mice by regulating the estrogen receptor ERα, promoting the production of NO, inhibiting the NF-κB signaling pathway, down-regulating the expression of MCP-1 and adhesion molecules; thus, having a potential therapeutic effect on atherosclerosis associated with estrogen deficiency.[42]

Overall, the above data suggest that diosgenin and its analogs can reduce endothelial inflammation and inhibit endothelial dysfunction by inhibiting leukocyte adhesion.

Inhibition of Platelet Aggregation and Prevention of Thrombosis

Atherosclerosis is rarely fatal. However, if it remains untreated, VSMCs will be progressively lost and foam cells will undergo apoptotic disintegration and release matrix metalloproteinases (MMPs), especially MMP-2 and −9, which gradually thins and degrades the fibrous cap, induces angiogenesis, and increases susceptibility to plaque rupture.[63] Plaque rupture leads to leakage of prethrombotic substances, inducing thrombosis at the site of rupture, which, in turn, may lead to fatal cardiovascular events. Therefore, the prevention of thrombosis helps reduce the occurrence of life-threatening cardiovascular and cerebrovascular events.

Endothelial cells express various molecules with anticoagulant, antiplatelet, and fibrinolytic properties, and their integrity is of great importance for clotting inhibition and thrombosis.[64] The plasminogen activator (PA) synthesized by endothelial cells promotes plasmin production and inhibits thrombosis. When stimulated or injured, endothelial cells can synthesize clotting substances, such as von Willebrand factor (vwF), platelet-activating factor (such as Par), and thromboxane (such as TXA) to promote platelet adhesion and aggregation on the damaged vascular wall. This then promotes the expression of tissue factor (TF), coagulation factor V, IX, X, etc., which facilitates blood coagulation and

blood flow obstruction.[65] In addition, plasminogen activator inhibitor-1 (PAI-1) in endothelium and platelets can inhibit PA expression and promotes atherosclerotic thrombosis.[66]

Diosgenin can reduce platelet aggregation rate, prolong prothrombin time (PT), thrombin time (TT) and activate partial thromboplastin time (APTT), inhibit thrombus formation, and increase the dissolution of blood clots in a dose-dependent manner, which, in turn, improve anticoagulant function in rats.[67] Besides, after structural modification of diosgenin, the anti-thrombotic effect of diosgenin is further enhanced with no side effects; moreover, the anti-thrombotic effect of diosgenin seems to be superior to that of asprin.

In vitro and in vivo studies have shown that prodrug micelles containing derivatives diosgenin and polyethylene glycol (PEG) can inhibit the adhesion, aggregation, apoptosis, and activation of platelets and regulate the APTT value by prolonging the activity of factor VIII (FVIII), which is involved in the intrinsic coagulation pathway, and increases the anti-thrombotic effect without causing excessive bleeding and obvious histological damage.[68]

Compound 5, a diosgenin derivative formed by substituting the carboxyl group in aspirin structure with diosgenin, shows a high inhibitory rate on platelet aggregation and could regulate the activation of FVIII and prolong APTT. Compared to aspirin, it has a lower risk of bleeding and fewer gastric mucosal lesions.[24] A similar effect was observed using compound 3 formed by combining the C3 position of diosgenin with a disaccharide consisting of glucose and galactose residues.[69] Therefore, these compounds may potentially be used as antiplatelet inhibitors.

Diosgenin can also inhibit the expression of PAI-1 and improve the endothelial dysfunction induced by palmitic acid. This mechanism may be related to the recovery of the anticoagulant effect of PA and the improvement of the endothelial pre-thrombotic state.[41] Moreover, NO produced by endothelial cells is also an important short-acting platelet inhibitor, and the regulation of NO by diosgenin and diosgenin may have a positive role in platelet aggregation and inhibition of thrombosis

To sum up, diosgenin and its analogs are pleiotropic drugs, which can regulate endothelial function, reduce endothelial dysfunction and inhibit atherosclerosis by regulating nitric oxide metabolism, monocyte adhesion, redox balance, leukocyte adhesion, hemostasis and thrombotic balance.

Regulation of the Function of Vascular Smooth Muscle

Inhibit Intimal Hyperplasia/Vascular Smooth Muscle Cell Proliferation and Migration

When stimulated by inflammatory factors, chemokines, and growth factors, VSMCs with contractile phenotype are transformed to cells with synthetic phenotype, which have more invasive, synthetic, and proliferative features. These cells migrate into the damaged vascular intima, where they proliferate and secrete a large amount of extracellular matrix and inflammatory factors (such as IL-1 and TNF-α), participating in the formation and development of fibrous caps in the early stage of atherosclerosis.[70] Smooth muscle cells (SMCs), together with the interstitial collagen and elastin, contribute to plaque stability to a certain extent. However, with the proliferation of SMC, the atherosclerotic plaque thickens can cause coronary artery stenosis and lead to a series of ischemic syndromes, such as hypertension, ischemic stroke, and renal impairment. Studies have suggested that diosgenin 10uM can inhibit the migration of VSMC by 45%, contraction by 25%, improve cell viability and calcium homeostasis, and further improve VSMC function.[71] Intimal hyperplasia is one of the key factors causing restenosis and atherosclerosis after percutaneous coronary angioplasty. Studies have shown that diosgenin and PD can inhibit proliferation, migration, and phenotypic transformation of VSMC and inhibit intima thickening in rat carotid artery balloon injury model through the inhibition of ERK1/2, FOXM1, and ADAM15 expression.[72,73] Therefore, Diosgenin is considered as a promising drug for the treatment of arteriosclerosis and restenosis after PCI.

Regulation of Vascular Calcification

Focal calcification gradually develops in atherosclerotic plaques with age, promoting plaque rupture.[74] Senescent apoptotic VSMCs are not easily removed and become the main source of calcification matrix, which promotes vascular calcification through conversion to osteoblast phenotype.[75] H_2O_2 can promote VSMC calcification, which is associated with increased activity and expression of Runt-related transcription factor 2 (Runx2).[76] Furthermore, Runx2 promotes the

differentiation and maturation of osteoblast and chondrocytes, thereby increasing vascular calcification.[77] Studies have shown that diosgenin can inhibit aortic VSMC phenotype changes and vascular calcification induced by renal failure via regulation of oxidative stress and reduction of the activity and expression of Runx2 by reducing H_2O_2.[37,51] Therefore, diosgenin may have an important role in vascular calcification. Above all, diosgenin and its analogs can inhibit the progression of atherosclerosis by inhibiting the proliferation, migration, and calcification of VSMCs.

Regulation of Lipid Metabolism

Dyslipidemia has an important role in atherosclerosis. The infiltration of plasma lipoproteins, free cholesterol (FC), cholesterol ester (CE), triglycerides (TG), phospholipids, and apolipoproteins, may all lead to atherosclerosis. Low-density lipoprotein cholesterol (LDL-C) is the most important and accepted risk factor for atherosclerosis.[78] On the other hand, high-density lipoprotein (HDL) promotes atherosclerotic plaque regression by promoting cholesterol outflow in foam cells and is negatively correlated with the occurrence and progression of atherosclerosis.[79]

Improvement of Hyperlipidemia

Numerous studies have shown that diosgenin, dioscin, tomatidine, dioscorea nipponica Makino, and *Sanyaku* containing diosgenin can induce total serum cholesterol (TC), very-low-density lipoprotein cholesterol (VLDL-C), LDL-C, TG, FTC, TTC and promote HDL-C levels in rats and mice[52,53,61,80–85] This mechanism is related to the up-regulation of peroxisome proliferation-activated receptor γ (PPARγ), cholesterol acyltransferase (LCAT), pancreatic lipase (PL), hepatic proteinase (HL), PGC1α -mediated lipoprotein lipase (LPL) and estrogen receptor α (ERα).[49,52,80–82,86]

In addition, sterol response element-binding proteins (SREBPs) are important lipid-derived transcription factors involved in cholesterol metabolism and lipid production.[87] Phosphorylation of AMPK inhibits lipid production and improves hepatic steatosis and atherosclerosis by inhibiting cleavage, nuclear translocation, and transcriptional activity of SREBP-1C and SREBP2.[88] Studies have shown that dioscin and diosgenin could inhibit the accumulation of fatty acids and triglycerides in HepG2 cells, AML12 cells, LO2 cells, 3T3-L1 cells and mouse liver and plasma by regulating the miR-125a-5p/STAT3 signaling pathway, the expression levels of AMPK, SREBP-1C, and downstream proteins related to lipid metabolisms, such as SCD, CPT, FAS, FoxO1, FASN, ACC, and ATGL, thereby reducing lipids improving the lipid profile.[89–95] Also, MPD, PD, and PPD can reduce the expression of genes related to triglyceride and cholesterol synthesis by inhibiting the levels of SREBP1c, SREBP2, and microRNA33a/b.[96,97] In addition, PPD has a significant therapeutic effect on estrogen-deficient atherosclerosis, and its therapeutic effect is comparable to that of 17β-estradiol.[98]

Liver X receptor (LXRs) is also an important factor regulating lipid metabolism, mediating the activation of SREBP-1c.[99] Diosgenin and yamogenin (dienantiomer of Diosgenin) have LXR-a antagonist effect; they can inhibit the upregulation of SREBP-1C induced by LXR-α agonist and LXR-α -mediated SREBP-1c induced by high-fat diet, and inhibit the accumulation of fat in plasma and liver cells. However, the activation effect of yamogenin on LXR is lower than that of diosgenin.[94,100,101] Also, LXRs may mediate the regulation of diosgenin on cholesterol homeostasis, yet, further studies are needed to confirm this data.

The proprotein convertase subtilisin-like/kexin type 9 (PCSK9) promotes LDLR degradation and has an important role in cholesterol homeostasis.[102] Studies have shown that MPD can promote the up-regulation of LDLR, reduce LDL-C level and improve atherosclerosis by inhibiting PCSK9.[96] Human acyl-CoA:cholesterol acyltransferase (ACAT), the only intracellular cholesterol esterification enzyme, is involved in the absorption, transport, and storage of cholesterol, and its overexpression is closely related to atherosclerosis.[103,104] Tomatidine can reduce serum LDL and cholesterol ester by inhibiting ACAT, inhibiting foam cell formation and alleviating atherosclerosis.[105]

Diosgenin can also promote the synthesis rate of liver cholesterol and improve hypercholesterolemia in rats via inhibition of the absorption of intestinal cholesterol by reducing intestinal surface area and reducing liver and plasma cholesterol.[106,107] The increased rate of liver cholesterol synthesis is partially due to the increased activity of HMG-COA reductase.[15,52,85,108] In addition, compared with atorvastatin treatment alone, the combination of diosgenin resulted in a higher rate of cholesterol reduction and an increase in neutral sterol excretion in the liver and other tissues of rats.[15,109] To sum up, diosgenin combined with statins may have a greater role in improving plasma cholesterol levels.

Inhibition of Plaque/Foam Cell Formation

The large lipid nuclei formed by foam cells are the hallmark feature of atherosclerosis. After being recruited to the intima, monocytes differentiate into macrophages, which ingest modified lipids through various pathways and become foam cells.[110] Foam cells not only secrete pro-inflammatory mediators but also lead to the formation of the necrotic core caused by macrophage apoptosis, which further promotes arterial wall inflammation and monocyte recruitment in advanced lesions, further aggravating atherosclerosis.[111] Therefore, inhibition of foam cell-mediated plaque formation is important in treating atherosclerosis.

Uncontrolled internalization of low-density lipoprotein promotes the formation of foam cells. Scavenger receptors (SRs), including SR-A, CD36, SR-BI, and LOX-1, further mediates the binding and internalization of oxLDL.[112] Dioscin can prevent dendritic cell activation and atherosclerotic plaque formation by inhibiting oxLDL uptake by inhibiting CD36, SR-A, LOX-1, and P38 MAPK expression.[113] It could also reduce cholesterol uptake of ox-LDL-treated macrophages through LOX-1 and decrease cholesterol levels in cells and aortic tissues, which leads to the inhibition of foam cell formation and atherosclerotic plaques.[83]

Efflux of cholesterol from macrophages is an important defense against foam cell formation, a process also known as reverse cholesterol transport (RCT). High-density lipoproteins promote macrophage cholesterol efflux by binding their apolipoprotein APOA-I to specific transporters of the ATP-binding cassette (ABC) gene family,[114] among which ABCA1 mediates approximately one-third of cholesterol efflux.[115]

In vivo and in vitro experiments demonstrated that diosgenin promotes ABCA1-mediated cholesterol excretion from macrophage foam cells, reduces cholesterol accumulation in macrophages and lipid deposition in the aorta, and alleviates atherosclerosis by inhibiting miR-19b expression.[116] Moreover, MPD, PD, and PPD can promote ABCA1-mediated cholesterol efflux by inhibiting the level of microRNA33a/b.[96,97]

Hence, diosgenin and its analogs can inhibit the formation of atherosclerotic plaques not only by regulating systemic lipid metabolism but also by improving local lipid absorption.

Inhibition of Intestinal Cholesterol Absorption/Promotion of Bile Cholesterol Excretion

Inducing plaque regression is often the best target for treating severe atherosclerosis. RCT can inhibit plaque progression and induce plaque regression. Besides RCT, the cholesterol transport system composed of the liver and intestine contributes to maintaining systemic cholesterol homeostasis and reducing atherosclerosis.[117] The cholesterol transport system not only inhibits the intestinal absorption of cholesterol but also carries cholesterol from peripheral tissues to the liver, excreting it into bile or forming free cholesterol, which is then excreted through the intestine to the body.[118] Diosgenin can promote fecal cholesterol excretion and inhibit cholesterol absorption in mice and rats;[85,107,119] this effect is related to Niemann-Pick C1-Like 1 (NPC1L1) and ATP-binding cassette G5/8(ABCG5/8). NPC1L1 is a transmembrane protein highly expressed in the intestine and liver, which mediates the excretion of intestinal sterols and hepatic cholesterol.[120] Li et al found that diosgenin could inhibit cholesterol absorption by down-regulating intestinal NPC1L1 expression.[85] However, Temel et al obtained different results, suggesting that diosgenin does not alter the expression of cholesterol transport-related genes (such as NPC1L1) in intestinal epithelial cells but stimulates fecal cholesterol excretion by regulating hepatic cholesterol metabolism.[107] Yet, this contradictory result may be related to different experimental animal models and detection methods.

ABCG5/8, as an important transporter in the intestine and liver, mediates the elimination of most bile cholesterol.[121] Previous studies found that diosgenin increases the cholesterol concentration in the bile of normal and cholesterol-fed rats by 4–15 times but does not affect the levels of bile acids, phospholipids, and bile salts.[15,122–125] Temel et al and Yu et al further suggested that cholesterol secretion is unrelated to liver ABCG5/8 expression.[107,126] These data were further confirmed by Kosters et al;[123] still, they also found no cholesterol secretion in ABCG8-/- mice treated with diosgenin, thus suggesting that ABCG8 activity is indispensable for diosgenin-mediated cholesterol excretion. Further studies showed that diosgenin could increase the transfer of cholesterol to heterodimer and promote bile cholesterol secretion through ABCG5/8 dependent and non-dependent pathways.[127] Besides, Li et al also found that diosgenin could inhibit LXR-α and increase the expression of ABCG5 and ABCG8 in the liver and intestine.[85] However, Kamisako et al found that diosgenin only affects the expression of ABCG5/8 in the liver but has no effect on the expression of ABCG5/8 in the

intestine.[128] In addition, diosgenin enhances the transport of peripheral cholesterol to the liver by promoting the expression of SRB1 and CES-1 and promoting the conversion of cholesterol into bile acid by stimulating the expression of CYP7A1 and FXR. It can also inhibit cholesterol absorption by increasing intestinal SRB1 and CES-1.[129]

Thus, it has been concluded that diosgenin and its analogs are effective cholesterol absorption inhibitors, affecting lipid metabolism through various pathways and alleviating hypercholesterolemia and atherosclerosis. Its mechanism is related to the regulation of lipase activity (LPL, PL, and HL), the expression of transcription factors related to lipid metabolism, the promotion of cholesterol outflow, the inhibition of intestinal absorption of cholesterol, and the increase of cholesterol secretion into bile. (As shown in Figure 2)

Inhibition of Inflammation

There is no doubt that the progressive narrowing of arterial lumen caused by hyperlipidemia is causally related to atherosclerosis. However, recent studies have proved that atherosclerosis may be characterized as chronic artery wall inflammation.[130]

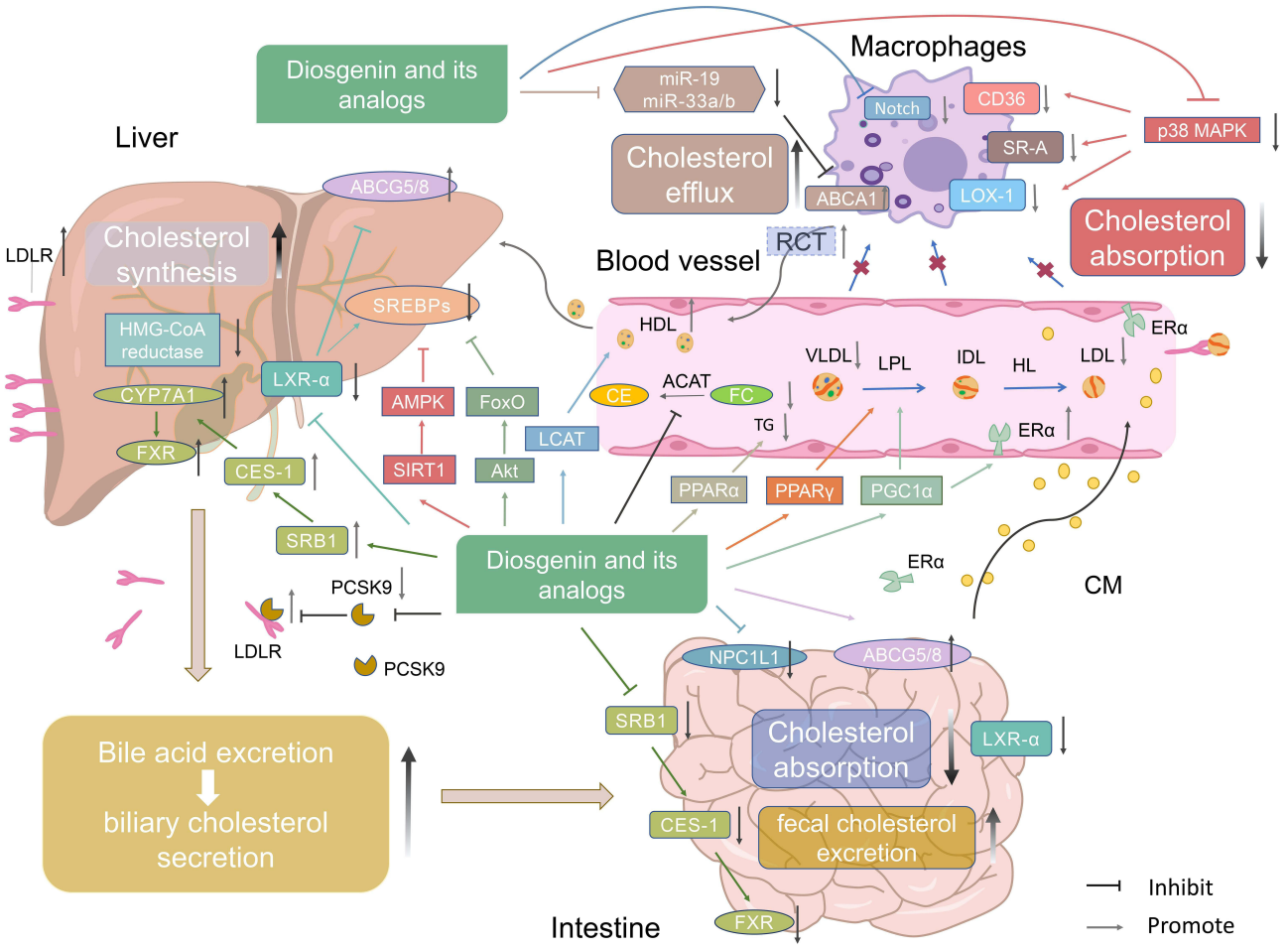

Figure 2 Mechanism of diosgenin and its analogs in lipid metabolism. Diosgenin and its analogs can affect plasma cholesterol, triglyceride, and fatty acid levels by inhibiting SREBPs and its downstream proteins related to lipid metabolism via regulation of SIRT1/AMPK signaling pathway, Akt/FoxO signaling pathway, LXR signaling pathway and inhibiting lipid metabolism enzymes (LPL, PL, HL). In addition, PPAR signaling pathway and PGC1α/ ERα signaling pathway also play important roles in plasma lipid metabolism. Diosgenin and its analogs can also inhibit hepatic cholesterol synthesis by inhibiting HMG-CoA reductase and increase HDL by improving LCAT. Among these analogs, tomatidine can inhibit cholesterol esterification by inhibiting ACAT, thereby reducing serum LDL and cholesterol esters. During local plaque cholesterol metabolism, diosgenin and its analogs also inhibit foam cell formation by inhibiting Notch signaling pathway and inhibit cholesterol intake by regulating scavenger receptors (SR-A, CD36 and LOX-1) via inhibition of p38 MAPK and promote cholesterol efflux mediated by ABCA1 via inhibition of the expression of miR-19/miR-33a/b. Diosgenin and its analogs can also promote the excretion of liver cholesterol, inhibit the absorption of intestinal cholesterol by promoting the expression of ABCG 5/8 and inhibiting the expression of NPC1L1 in the liver and intestine. In addition, diosgenin can promote the CES-1/SRB1/ CYP7A1/ FXR signaling pathway in the liver, which promote the transformation of cholesterol into bile acids and excretion from feces, and can also decrease CES-1, SRB1 and FXR in the intestine, which inhibits cholesterol absorption. At the same time, MPD and PD promote the up-regulation of LDLR and the transport of circulating cholesterol to the liver by inhibiting PCSK9.

As previously mentioned, endothelial cells are activated by inflammatory signals in the early stage of atherosclerosis. These cells express various adhesion molecules that promote leukocyte adhesion, further stimulating inflammation and plaque progression. Diosgenin and its analogs can inhibit the expression of adhesion molecules, inhibit the adhesion of leukocytes to endothelium, and reduce inflammation. In addition to regulating the expression of adhesion molecules, in vitro studies showed that diosgenin can inhibit PA-stimulated HUVEC inflammation (TNF-α, IL-6) through the IKKβ/NF-kB pathway.[41] It also promotes the expression of adiponectin and PPARγ with anti-inflammatory effects by inhibiting IKKβ/NF-κB signaling pathway and increasing AMPK activity in PAT, which then inhibits PAT inflammation and alleviates endothelial inflammation through a paracrine or endocrine pathway.[40]

As atherosclerosis progresses, macrophages enter the intima and ingest large amounts of LDL-C, promoting the formation of foam cells and the deposition of lipids on the inner walls of blood vessels. During this process, the pro-inflammatory M1-like macrophages of the disease-modifying macrophages have an important role in inflammation by secreting various chemokines (eg, MCP-1) and cytokines (eg, IL-12, IL-1, TNFα), which further activate the endothelium and lead to additional rounds of monocyte recruitment.[131,132] In addition, M1 macrophages are the dominant phenotype in the shoulder region of the plaque prone to rupture; they degrade the extracellular matrix in the plaque by increasing the secretion of MMPs, increasing plaque instability and vulnerability to rupture. At the same time, M2 macrophages have a dominant role in scar stability[133] and can promote the alleviation of plaque inflammation by secreting anti-inflammatory cytokines, such as IL-10.

Studies have shown that diosgenin can promote the differentiation of ox-LDL-induced monocytes and monocytes from the aorta into M2 macrophages, as evidenced by the increased expression of M2-specific chemokines (such as MDC, BLC, MIP-1α, etc.), which, in turn, reduces the expression of inflammatory mediators.[61] High expression of Arg-1 and AMPK promotes M2 differentiation and IL-10 secretion.[134] Diosgenin can also promote the polarization of M2 macrophages by stimulating the expression of Arg-1 and AMPK.[40] It also inhibits the differentiation of macrophages and alleviates atherosclerosis by inhibiting nuclear translocation of the Notch intracellular domain (NICD).[135] In addition to inhibiting the conversion of pro-inflammatory phenotypes, dioscin and diosgenin also inhibit the induction of inflammation in macrophages by pro-inflammatory mediators. For example, dioscin can inhibit the expression of inflammatory cytokines in THP-1 macrophages stimulated by LPS and Pam3CSK4 via downregulation of the TLR2/MyD88/NF-κB signaling pathway.[136] Besides, diosgenin can reduce the expression of inflammatory mediators in LPS/IFN-γ-induced RAW264.7 macrophages by inhibiting the activation of NF-κB, CK2, JNK, and AP-1,[137] and also reduce the free fatty acid-induced macrophage cellular inflammation.[138] Similarly, tomatidine can inhibit the expression of proinflammatory enzyme COX-2 and iNOS through suppressing NF-κB and JNK pathways in RAW 264.7cells, which in turns inhibits macrophage cellular inflammation.[139]

In addition to endothelial cells and macrophages, various cells, especially antigen-presenting cells in the adaptive immune response, have an important role in the progression of plaque inflammation. Dendritic cells are key mediators of antigen presentation, which can activate T cells in plaque and promote the progression of plaque inflammation. In vitro studies showed that diosgenin inhibits the secretion of pro-inflammatory cytokines IL-6 and IL-12 in dendritic cells and promotes the expression of IL-10. It is speculated that diosgenin may inhibit the activation of T cells, reduce vascular inflammation, and inhibit atherosclerosis by decreasing the maturation of dendritic cells and improving immune function.[113] Other immune cells, such as B cells, T cells, and neutrophils, promote inflammation in plaques; still, the role of diosgenin and its analogs in the immune regulation of atherosclerosis needs to be further examined.

During plaque progression, VSMCs migrate into the intima and secretes adhesion molecules to recruit monocytes into the intima. Diosgenin can inhibit the expression of TNF-α - induced vascular smooth muscle cell adhesion molecules and reduce macrophage adhesion to VSMCs by inhibiting MAPK/Akt/NF-κB signaling pathway.[140] In advanced atherosclerosis, inflammatory mediators such as IL-1β and TNF-α promote the release of matrix metalloproteinases, which degrade extracellular matrix components and contribute to plaque rupture.[141] They also promote tissue factor (TF) expression and thrombogenesis.[142] Diosgenin can inhibit the expression of TF in THP-1 monocytes induced by TNF-α by inhibiting the activation of NF-κB, Akt, and MAPK signaling pathways; it can also inhibit the procoagulant activity of TF, which is expected to prevent inflammation-induced thrombosis in the late stage of atherosclerosis.[143] Furthermore, in vivo studies have confirmed that diosgenin and its analogs have positive anti-inflammatory effects. For example, dioscin

and diosgenin can inhibit the nuclear translocation of NFκB and reduce the expressions of IL-6, IL-12, TNF-α, CYP2E1, COX-2, and HMGB1 in the plasma, liver, and heart of Wistar and SD rats, C57BL/6J mice, and ob/ob mice induced by atherogenic diet, as well as pig models of coronary heart disease.[39,54,83,91] Hence, diosgenin and its analogs are very effective inflammatory inhibitors, acting on various stages of the progression of atherosclerosis. The mechanism is related to the inhibition of the adhesion of pro-inflammatory leukocytes, promoting the phenotypic transformation of anti-inflammatory macrophages, down-regulation of the NF-κB signaling pathway related to inflammation, and inhibition of the expression of inflammatory cytokines.

Pharmacokinetics and Bioavailability

Although the above data show an exciting anti-atherosclerotic effect of diosgenin and its analogs, scarce information regarding its pharmacokinetics and bioavailability limits its clinical research and development.

Diosgenin is a strong hydrophobic compound with poor pharmacokinetic parameters (LogP; 5.7).[144] Compared with diosgenin, dioscin shows better intestinal permeability but higher instability in gastric and intestinal juices, which results in very low bioavailability;[145] its absolute oral bioavailability is only about 0.2% (vs bioavailability of diosgenin is 6%) with a long half-life (t1/2) and weak absorption of drugs by the enterohepatic recycling.[146,147] Both products are mainly excreted through feces.

Recently, several methods have been used to improve its bioavailability. For example, Okawara et al combined diosgenin with β-cyclodextrin (β-CD) to form an inclusion complex, improving the product bioavailability to 45%.[146] Furthermore, the interaction of liquid crystals with β -cyclodextrin further improves the solubility and bioavailability of diosgenin.[148] However, their preparation is time-consuming and requires many excipients, and the drug loading is low. Therefore, another delivery system that is easier to manufacture is needed to improve the drug load. Kim et al improved hydrophobicity, poor pharmacokinetic parameters, and enhanced bioavailability by conjugating diosgenin with a hydrophilic unit, tetraethylene glycol.[149] Therefore, replacing the glycosylated group with small molecular weight polyethylene glycol (PEG) may be a suitable strategy to improve the pharmacokinetic parameters of diosgenin. Moreover, nanocrystalline preparation can be used for drugs with extremely low aqueous solubility and high logP, increasing the adhesion of drugs in the gastrointestinal tract and achieving high drug loading.[150,151] Research indicated that the development of nanocrystals could improve the dissolution rate and oral bioavailability and the biological effects of diosgenin.[152] As mentioned earlier, diosgenin prodrug nanoparticles can prevent thrombosis with no risk of bleeding. However, nanomaterials may lead to the destruction of heat-sensitive components. Pan et al showed that diosgenin content in 50 mesh-size Dioscorea pseudojaponica (DP) was higher than in nanoscale DP. They also found a greater lipid-lowering effect, which may be mediated by the AMPK-ACC pathway.[153]

In addition, the development of diosgenin derivatives is beneficial for improving solubility, permeability, and biological activity. For example, compound 5, a diosgenin derivative mentioned earlier, showed better anti-thrombotic properties.[24] Diosgenin derivatives containing primary amine not only enhance the permeability but also inhibit the production of ROS, iNOS, COX-2, and pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) in lipopolysaccharide (LPS)-stimulated microglia through NF-κB and JNK MAPK signaling pathways and promote NO synthesis, which has an anti-inflammatory and antioxidant stress role.[154] In addition, the bioactivity of diosgenin can be improved by covalent modification.[155] For example, Laura et al improved the anti-proliferation and anti-oxidation properties of diosgenin by combining thio- and selenoureas at the C-3 position.[156] These data suggest that structural modification of dioscin and diosgenin and the development of new drug delivery systems and derivatives may improve the bioavailability and enhance the anti-atherosclerosis efficacy.

Conclusions and Perspectives

In general, diosgenin and its analogs show potential therapeutic effects on atherosclerosis. They can improve endothelial dysfunction by regulating vascular tension, oxidative stress, leukocyte adhesion, platelet aggregation, and thrombosis. They can also inhibit the proliferation, migration, and calcification of VSMCs by regulating VSMC phenotype conversion, improve lipid metabolism by inhibiting foam cell formation, regulating hyperlipidemia, inhibiting intestinal cholesterol absorption and promoting bile cholesterol excretion. These molecules have a positive regulatory effect on

various inflammatory cell types, inflammatory signaling pathways, and inflammatory cytokines involved in various stages of AS. (As shown in Figure 3) Besides, diosgenin and its analogs have also shown promising results in treating hyperlipidemia, diabetes, non-alcoholic fatty liver, obesity, and other metabolic syndromes.[157] We summarized the roles of diosgenin, dioscin, and other analogs of diosgenin in atherosclerosis-related diseases in Supplementary Tables 1–3 respectively respectively.

Statins, a gold standard product for the treatment of atherosclerosis, have also shown some shortcomings in lowering cholesterol. For example, statins can only reduce cholesterol levels by inhibiting its synthesis but have little effect on inhibiting intestinal cholesterol absorption and promoting its excretion.[158] Inflammatory inhibitors against atherosclerosis have also not been commercialized due to their high cost. Diosgenin and its analogs significantly inhibit cholesterol absorption and promote its excretion and reduce hypercholesterolemia. They are aslo widely available and are low cost. Therefore, diosgenin and its analogs are expected to become a new alternative anti- atherosclerosis drug. Yet, poor pharmacokinetic profile, low bioavailability, low aqueous solubility and instability in the gastrointestinal tract limit their clinical application. Thus, more information on the pharmacokinetics and metabolism of diosgenin and its analogs is required. In addition, it is necessary to develop more efficient drug delivery systems and more safe and effective derivatives to improve their bioavailability.

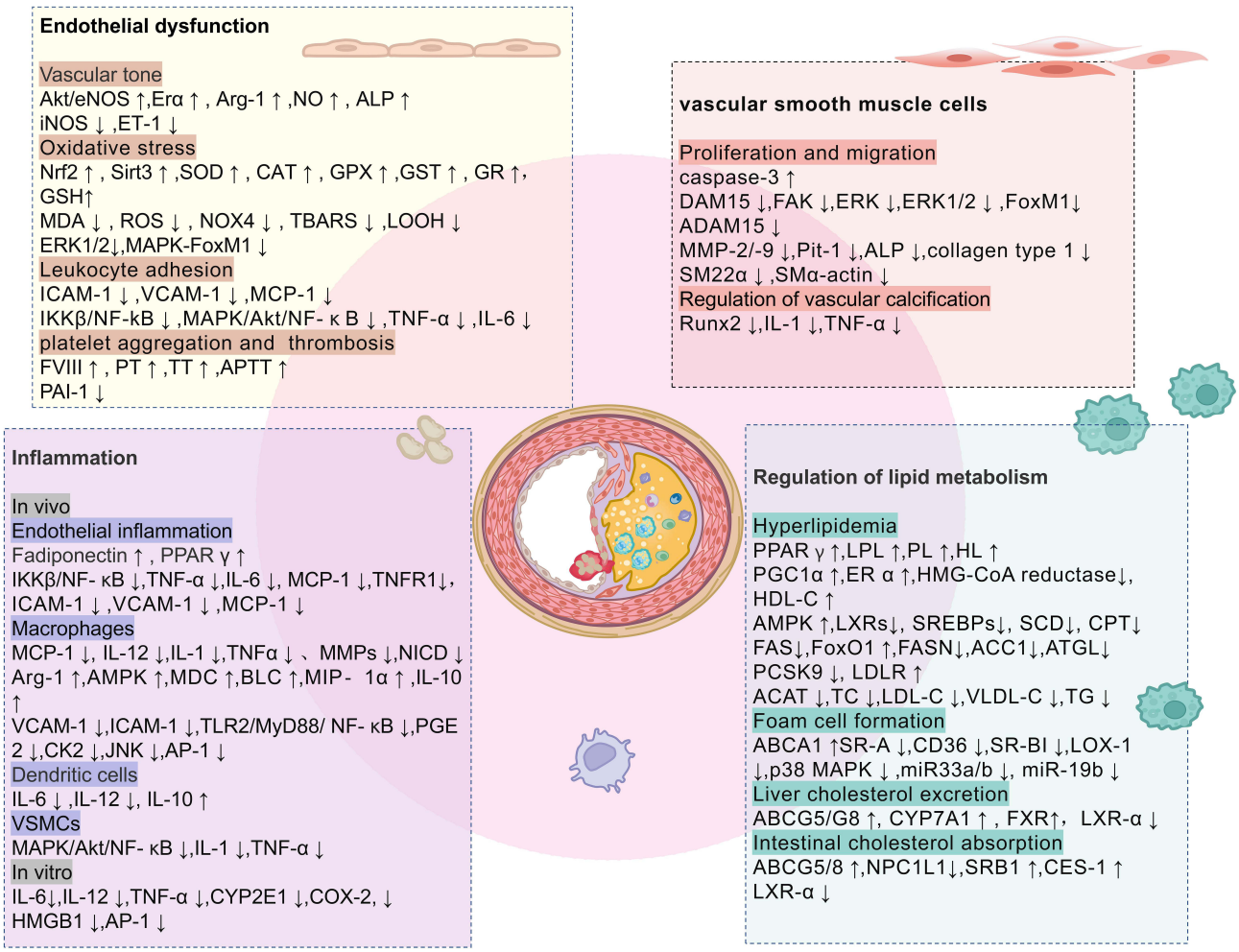

Figure 3 Mechanism of diosgenin and its analogs in regulating atherosclerosis. Diosgenin and its analogs improve AS by regulating endothelial dysfunction (eg, vascular tension, oxidative stress, leukocyte adhesion, platelet aggregation, and thrombosis), inhibiting proliferation, migration, and calcification of VSMCs, regulating lipid metabolism (eg, ameliorating hyperlipidemia, inhibiting foam cell formation, promoting liver cholesterol excretion, and inhibiting intestinal cholesterol absorption), and inhibiting inflammatory processes.

Studies on diosgenin and its analogs in atherosclerosis and their effect on immune regulation are still lacking, while studies reporting on other analogs are just in the infancy stage. Parallel comparisons of biological activities, pharmacodynamic properties, and content differences between species among multiple structural analogs are also necessary. What's more, although some clinical trials show that extractive preparations rich in diosgenin and its analogs, such as wild yam,[159] fenugreek,[160,161] Di'ao Xin Xue Kang capsule,[162] etc., have a positive effect on atherosclerosis, clinical trials diosgenin and its analogs are still lacking. In addition, it should be noted that whether these drugs exert their effects through diosgenin or other monomeric analogs alone or drug-drug interactions needs to be further explored. The translation of diosgenin and its analogs to the clinic needs to be strengthened in the future. Research on efficacy, dosage, adverse reactions, and drug interactions is also necessary.

Looking into the future, we believe that natural medicines will provide a broader space for new drug development. In 2015, Dr. Youyou Tu won the Nobel Prize in Physiology or Medicine for the extraction of artemisinin from Artemisia annua, a Chinese herbal medicine, which once again demonstrated the appeal and feasibility of treating patients with active ingredients derived from natural products. Therefore, we believe that researcher will recognize great potential of diosgenin and its analogs in developing new agents against atherosclerosis, which are also expected to have an important role in treating cardiovascular diseases in the future.

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Disclosure

All the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. World health statistics 2021: monitoring health for the SDGs, sustainable development goals. Available from: https://www.who.int/publications/i/item/9789240027053. Accessed July 12, 2022.
2. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*. 2011;17(11):1410–1422. doi:10.1038/nm.2538
3. Libby P, Buring JE, Badimon L, et al. Atherosclerosis. *Nat Rev Dis Primers*. 2019;5(1):56. doi:10.1038/s41572-019-0106-z
4. Tomaszewski M, Stepien KM, Tomaszewska J, Czuczwar SJ. Statin-induced myopathies. *Pharmacol Rep*. 2011;63(4):859–866. doi:10.1016/S1734-1140(11)70601-6
5. Sostres C, Lanas A. Gastrointestinal effects of aspirin. *Nat Rev Gastroenterol Hepatol*. 2011;8(7):385–394. doi:10.1038/nrgastro.2011.97
6. Cai G, Zhou W, Lu Y, Chen P, Lu Z, Fu Y. Aspirin resistance and other aspirin-related concerns. *Neurol Sci*. 2016;37(2):181–189. doi:10.1007/s10072-015-2412-x
7. Yu Y, Hu S, Li G, et al. Comparative effectiveness of Di'ao Xin Xue Kang capsule and compound Danshen tablet in patients with symptomatic chronic stable angina. *Sci Rep*. 2014;4(1):7058. doi:10.1038/srep07058
8. Wang LY, Tang JY, Liu J, et al. Dynamic changes in phenotypic groups in patients with stable angina pectoris after treatment with Xinxuekang capsule: a randomized controlled trial. *Curr Vasc Pharmacol*. 2015;13(4):492–503. doi:10.2174/1570161112666141014151858
9. Jia Y, Chen C, Ng CS, Leung SW. Meta-analysis of randomized controlled trials on the efficacy of Di'ao Xinxuekang capsule and isosorbide dinitrate in treating angina pectoris. *Evid Based Complement Alternat Med*. 2012;2012:904147. doi:10.1155/2012/904147
10. Chen Y, Tang YM, Yu SL, et al. Advances in the pharmacological activities and mechanisms of diosgenin. *Chin J Nat Med*. 2015;13(8):578–587. doi:10.1016/S1875-5364(15)30053-4
11. Li X, Liu S, Qu L, et al. Dioscin and diosgenin: insights into their potential protective effects in cardiac diseases. *J Ethnopharmacol*. 2021;274:114018. doi:10.1016/j.jep.2021.114018
12. Russell L, Hicks GS, Low AK, Shepherd JM, Brown CA. Phytoestrogens: a viable option? *Am J Med Sci*. 2002;324(4):185–188. doi:10.1097/00000441-200210000-00004
13. National Center for Biotechnology Information, PubChem database. Dioscin, CID=119245. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Dioscin. Accessed July 12, 2022.
14. Sun F, Yang X, Ma C, et al. The effects of diosgenin on hypolipidemia and its underlying mechanism: a review. *Diabetes Metab Syndr Obes*. 2021;14:4015–4030. doi:10.2147/DMSO.S326054
15. Cayen MN, Dvornik D. Effect of diosgenin on lipid metabolism in rats. *J Lipid Res*. 1979;20(2):162–174. doi:10.1016/S0022-2275(20)40628-5

16. Laguna J, Gomez-Puyou A, Pena A, Guzman-Garcia J. Effect of diosgenin on cholesterol metabolism. *J Atheroscler Res*. 1962;2(6):459–470. doi:10.1016/S0368-1319(62)80017-9
17. Ondevilla JC, Hanashima S, Mukogawa A, Umegawa Y, Murata M. Diosgenin-induced physicochemical effects on phospholipid bilayers in comparison with cholesterol. *Bioorg Med Chem Lett*. 2021;36:127816. doi:10.1016/j.bmcl.2021.127816
18. Zhang C, Peng J, Wu S, et al. Dioscin promotes osteoblastic proliferation and differentiation via Lrp5 and ER pathway in mouse and human osteoblast-like cell lines. *J Biomed Sci*. 2014;21(1):30. doi:10.1186/1423-0127-21-30
19. Au AL, Kwok CC, Lee AT, et al. Activation of iberiotoxin-sensitive, Ca2+-activated K+ channels of porcine isolated left anterior descending coronary artery by diosgenin. *Eur J Pharmacol*. 2004;502(1–2):123–133. doi:10.1016/j.ejphar.2004.08.045
20. Jesus M, Martins AP, Gallardo E, Silvestre S. Diosgenin: recent highlights on pharmacology and analytical methodology. *J Anal Methods Chem*. 2016;2016:4156293. doi:10.1155/2016/4156293
21. Xu T, Zhang S, Zheng L, Yin L, Xu L, Peng J. A 90-day subchronic toxicological assessment of dioscin, a natural steroid saponin, in Sprague-Dawley rats. *Food Chem Toxicol*. 2012;50(5):1279–1287. doi:10.1016/j.fct.2012.02.027
22. Xu T, Zheng L, Xu L, et al. Protective effects of dioscin against alcohol-induced liver injury. *Arch Toxicol*. 2014;88(3):739–753. doi:10.1007/s00204-013-1148-8
23. Tohda C, Yang X, Matsui M, et al. Diosgenin-rich yam extract enhances cognitive function: a placebo-controlled, randomized, double-blind, crossover study of healthy adults. *Nutrients*. 2017;9(10):10. doi:10.3390/nu9101160
24. Zheng H, Wei Z, Xin G, et al. Preventive effect of a novel diosgenin derivative on arterial and venous thrombosis in vivo. *Bioorg Med Chem Lett*. 2016;26(14):3364–3369. doi:10.1016/j.bmcl.2016.05.032
25. Qin Y, Wu X, Huang W, et al. Acute toxicity and sub-chronic toxicity of steroidal saponins from Dioscorea zingiberensis C.H.Wright in rodents. *J Ethnopharmacol*. 2009;126(3):543–550. doi:10.1016/j.jep.2009.08.047
26. Bloomer RJ. Decreased blood antioxidant capacity and increased lipid peroxidation in young cigarette smokers compared to nonsmokers: impact of dietary intake. *Nutr J*. 2007;6(1):39. doi:10.1186/1475-2891-6-39
27. Frostegard J, Wu R, Lemne C, Thulin T, Witztum JL, de Faire U. Circulating oxidized low-density lipoprotein is increased in hypertension. *Clin Sci (Lond)*. 2003;105(5):615–620. doi:10.1042/CS20030152
28. Zhou MS, Chadipiralla K, Mendez AJ, et al. Nicotine potentiates proatherogenic effects of oxLDL by stimulating and upregulating macrophage CD36 signaling. *Am J Physiol Heart Circ Physiol*. 2013;305(4):H563–574. doi:10.1152/ajpheart.00042.2013
29. Furieri LB, Galan M, Avendano MS, et al. Endothelial dysfunction of rat coronary arteries after exposure to low concentrations of mercury is dependent on reactive oxygen species. *Br J Pharmacol*. 2011;162(8):1819–1831. doi:10.1111/j.1476-5381.2011.01203.x
30. Xu S, Ilyas I, Little PJ, et al. Endothelial dysfunction in atherosclerotic cardiovascular diseases and beyond: from mechanism to pharmacotherapies. *Pharmacol Rev*. 2021;73(3):924–967. doi:10.1124/pharmrev.120.000096
31. Sausbier M, Schubert R, Voigt V, et al. Mechanisms of NO/cGMP-dependent vasorelaxation. *Circ Res*. 2000;87(9):825–830. doi:10.1161/01.RES.87.9.825
32. Ricciardolo FL, Nijkamp FP, Folkerts G. Nitric oxide synthase (NOS) as therapeutic target for asthma and chronic obstructive pulmonary disease. *Curr Drug Targets*. 2006;7(6):721–735. doi:10.2174/138945006777435290
33. Tian J, Yan Z, Wu Y, et al. Inhibition of iNOS protects endothelial-dependent vasodilation in aged rats. *Acta Pharmacol Sin*. 2010;31(10):1324–1328. doi:10.1038/aps.2010.111
34. Ignarro LJ, Napoli C. Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis. *Curr Diab Rep*. 2005;5(1):17–23. doi:10.1007/s11892-005-0062-8
35. Kim F, Tysseling KA, Rice J, et al. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. *Arterioscler Thromb Vasc Biol*. 2005;25(5):989–994. doi:10.1161/01.ATV.0000160549.60980.a8
36. Pernow J, Shemyakin A, Bohm F. New perspectives on endothelin-1 in atherosclerosis and diabetes mellitus. *Life Sci*. 2012;91(13–14):507–516. doi:10.1016/j.lfs.2012.03.029
37. Manivannan J, Shanthakumar J, Arunagiri P, Raja B, Balamurugan E. Diosgenin interferes coronary vasoconstriction and inhibits osteochondrogenic transdifferentiation of aortic VSMC in CRF rats. *Biochimie*. 2014;102:183–187. doi:10.1016/j.biochi.2014.03.011
38. Szabo K, Gesztelyi R, Lampe N, et al. Fenugreek (Trigonella Foenum-Graecum) seed flour and diosgenin preserve endothelium-dependent arterial relaxation in a rat model of early-stage metabolic syndrome. *Int J Mol Sci*. 2018;19(3):798. doi:10.3390/ijms19030798
39. Binesh A, Devaraj SN, Halagowder D. Atherogenic diet induced lipid accumulation induced NFkappaB level in heart, liver and brain of Wistar rat and diosgenin as an anti-inflammatory agent. *Life Sci*. 2018;196:28–37. doi:10.1016/j.lfs.2018.01.012
40. Chen Y, Xu X, Zhang Y, et al. Diosgenin regulates adipokine expression in perivascular adipose tissue and ameliorates endothelial dysfunction via regulation of AMPK. *J Steroid Biochem Mol Biol*. 2016;155(Pt A):155–165. doi:10.1016/j.jsbmb.2015.07.005
41. Liu K, Zhao W, Gao X, Huang F, Kou J, Liu B. Diosgenin ameliorates palmitate-induced endothelial dysfunction and insulin resistance via blocking IKKbeta and IRS-1 pathways. *Atherosclerosis*. 2012;223(2):350–358. doi:10.1016/j.atherosclerosis.2012.06.012
42. Sun B, Yang D, Yin YZ, Xiao J. Estrogenic and anti-inflammatory effects of pseudoprotodioscin in atherosclerosis-prone mice: insights into endothelial cells and perivascular adipose tissues. *Eur J Pharmacol*. 2020;869:172887. doi:10.1016/j.ejphar.2019.172887
43. Szasz T, Webb RC. Perivascular adipose tissue: more than just structural support. *Clin Sci (Lond)*. 2012;122(1):1–12. doi:10.1042/CS20110151
44. Oriowo MA. Perivascular adipose tissue, vascular reactivity and hypertension. *Med Princ Pract*. 2015;24(Suppl 1):29–37. doi:10.1159/000356380
45. Cheng KK, Lam KS, Wang Y, et al. Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. *Diabetes*. 2007;56(5):1387–1394. doi:10.2337/db06-1580
46. Wang Y, Branicky R, Noe A, Hekimi S. Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol*. 2018;217(6):1915–1928. doi:10.1083/jcb.201708007
47. Frank L, Massaro D. Oxygen toxicity. *Am J Med*. 1980;69(1):117–126. doi:10.1016/0002-9343(80)90509-4
48. Li H, Xie YH, Yang Q, et al. Cardioprotective effect of paeonol and danshensu combination on isoproterenol-induced myocardial injury in rats. *PLoS One*. 2012;7(11):e48872. doi:10.1371/journal.pone.0048872
49. Gong G, Qin Y, Huang W, et al. Protective effects of diosgenin in the hyperlipidemic rat model and in human vascular endothelial cells against hydrogen peroxide-induced apoptosis. *Chem Biol Interact*. 2010;184(3):366–375. doi:10.1016/j.cbi.2010.02.005

50. Pari L, Monisha P, Mohamed Jalaludeen A. Beneficial role of diosgenin on oxidative stress in aorta of streptozotocin induced diabetic rats. *Eur J Pharmacol*. 2012;691(1–3):143–150. doi:10.1016/j.ejphar.2012.06.038
51. Manivannan J, Barathkumar TR, Sivasubramanian J, Arunagiri P, Raja B, Balamurugan E. Diosgenin attenuates vascular calcification in chronic renal failure rats. *Mol Cell Biochem*. 2013;378(1–2):9–18. doi:10.1007/s11010-013-1588-8
52. Manivannan J, Balamurugan E, Silambarasan T, Raja B. Diosgenin improves vascular function by increasing aortic eNOS expression, normalize dyslipidemia and ACE activity in chronic renal failure rats. *Mol Cell Biochem*. 2013;384(1–2):113–120. doi:10.1007/s11010-013-1788-2
53. Son IS, Kim JH, Sohn HY, Son KH, Kim JS, Kwon CS. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (Dioscorea spp.), on high-cholesterol fed rats. *Biosci Biotechnol Biochem*. 2007;71(12):3063–3071. doi:10.1271/bbb.70472
54. Yang B, Xu B, Zhao H, et al. Dioscin protects against coronary heart disease by reducing oxidative stress and inflammation via Sirt1/Nrf2 and p38 MAPK pathways. *Mol Med Rep*. 2018;18(1):973–980. doi:10.3892/mmr.2018.9024
55. Jayachandran KS, Vasanthi HR, Rajamanickam GV. Antilipoperoxidative and membrane stabilizing effect of diosgenin, in experimentally induced myocardial infarction. *Mol Cell Biochem*. 2009;327(1–2):203–210. doi:10.1007/s11010-009-0058-9
56. Ratliff BB, Abdulmahdi W, Pawar R, Wolin MS. Oxidant mechanisms in renal injury and disease. *Antioxid Redox Signal*. 2016;25(3):119–146. doi:10.1089/ars.2016.6665
57. Jaiswal AK. Nrf2 signaling in coordinated activation of antioxidant gene expression. *Free Radic Biol Med*. 2004;36(10):1199–1207. doi:10.1016/j.freeradbiomed.2004.02.074
58. Qiao Y, Xu L, Tao X, et al. Protective effects of dioscin against fructose-induced renal damage via adjusting Sirt3-mediated oxidative stress, fibrosis, lipid metabolism and inflammation. *Toxicol Lett*. 2018;284:37–45. doi:10.1016/j.toxlet.2017.11.031
59. Yin M, Li C, Jiang J, et al. Cell adhesion molecule-mediated therapeutic strategies in atherosclerosis: from a biological basis and molecular mechanism to drug delivery nanosystems. *Biochem Pharmacol*. 2021;186:114471. doi:10.1016/j.bcp.2021.114471
60. Wu S, Xu H, Peng J, et al. Potent anti-inflammatory effect of dioscin mediated by suppression of TNF-alpha-induced VCAM-1, ICAM-1and EL expression via the NF-kappaB pathway. *Biochimie*. 2015;110:62–72. doi:10.1016/j.biochi.2014.12.022
61. Binesh A, Devaraj SN, Devaraj H. Expression of chemokines in macrophage polarization and downregulation of NFkappaB in aorta allow macrophage polarization by diosgenin in atherosclerosis. *J Biochem Mol Toxicol*. 2020;34(2):e22422. doi:10.1002/jbt.22422
62. Yang WS, Moon SY, Lee MJ, Lee EK, Park SK. Diosgenin, an activator of 1,25D3-MARRS receptor/ERp57, attenuates the effects of TNF-alpha by causing ADAM10-Dependent ectodomain shedding of TNF Receptor 1. *Cell Physiol Biochem*. 2017;43(6):2434–2445. doi:10.1159/000484396
63. Newby AC. Metalloproteinases and vulnerable atherosclerotic plaques. *Trends Cardiovasc Med*. 2007;17(8):253–258. doi:10.1016/j.tcm.2007.09.001
64. Badimon L, Vilahur G. Thrombosis formation on atherosclerotic lesions and plaque rupture. *J Intern Med*. 2014;276(6):618–632. doi:10.1111/joim.12296
65. van Loon JE, Kavousi M, Leebeek FW, et al. von Willebrand factor plasma levels, genetic variations and coronary heart disease in an older population. *J Thromb Haemost*. 2012;10(7):1262–1269. doi:10.1111/j.1538-7836.2012.04771.x
66. Vaughan DE. PAI-1 and atherothrombosis. *J Thromb Haemost*. 2005;3(8):1879–1883. doi:10.1111/j.1538-7836.2005.01420.x
67. Gong G, Qin Y, Huang W. Anti-thrombosis effect of diosgenin extract from Dioscorea zingiberensis C.H. Wright in vitro and in vivo. *Phytomedicine*. 2011;18(6):458–463. doi:10.1016/j.phymed.2010.08.015
68. Wei Z, Xin G, Wang H, et al. The diosgenin prodrug nanoparticles with pH-responsive as a drug delivery system uniquely prevents thrombosis without increased bleeding risk. *Nanomedicine*. 2018;14(3):673–684. doi:10.1016/j.nano.2017.12.019
69. Zhang R, Huang B, Du D, et al. Anti-thrombosis effect of diosgenyl saponins in vitro and in vivo. *Steroids*. 2013;78(11):1064–1070. doi:10.1016/j.steroids.2013.07.003
70. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev*. 2004;84(3):767–801. doi:10.1152/physrev.00041.2003
71. Esfandiarei M, Lam JT, Yazdi SA, et al. Diosgenin modulates vascular smooth muscle cell function by regulating cell viability, migration, and calcium homeostasis. *J Pharmacol Exp Ther*. 2011;336(3):925–939. doi:10.1124/jpet.110.172684
72. Fan T, He J, Yin Y, et al. Dioscin inhibits intimal hyperplasia in rat carotid artery balloon injury model through inhibition of the MAPK-FoxM1 pathway. *Eur J Pharmacol*. 2019;854:213–223. doi:10.1016/j.ejphar.2019.03.050
73. Chung YL, Pan CH, Wang CC, et al. Methyl protodioscin, a steroidal saponin, inhibits neointima formation in vitro and in vivo. *J Nat Prod*. 2016;79(6):1635–1644. doi:10.1021/acs.jnatprod.6b00217
74. Hruska KA. Vascular smooth muscle cells in the pathogenesis of vascular calcification. *Circ Res*. 2009;104(6):710–711. doi:10.1161/CIRCRESAHA.109.195487
75. Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL. Apoptosis regulates human vascular calcification in vitro: evidence for initiation of vascular calcification by apoptotic bodies. *Circ Res*. 2000;87(11):1055–1062. doi:10.1161/01.RES.87.11.1055
76. Byon CH, Javed A, Dai Q, et al. Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *J Biol Chem*. 2008;283(22):15319–15327. doi:10.1074/jbc.M800021200
77. Komori T. Regulation of proliferation, differentiation and functions of osteoblasts by Runx2. *Int J Mol Sci*. 2019;20(7):1694. doi:10.3390/ijms20071694
78. Albertini R, Moratti R, De Luca G. Oxidation of low-density lipoprotein in atherosclerosis from basic biochemistry to clinical studies. *Curr Mol Med*. 2002;2(6):579–592. doi:10.2174/1566524023362177
79. Murphy AJ, Westerterp M, Yvan-Charvet L, Tall AR. Anti-atherogenic mechanisms of high density lipoprotein: effects on myeloid cells. *Biochim Biophys Acta*. 2012;1821(3):513–521. doi:10.1016/j.bbalip.2011.08.003
80. Hashidume T, Sasaki K, Hirata J, et al. Effects of sanyaku and its constituent diosgenin on the fasted and postprandial hypertriacylglycerolemia in high-fat-diet-fed KK- A (y) mice. *J Agric Food Chem*. 2018;66(38):9968–9975. doi:10.1021/acs.jafc.8b03040
81. Yang Q, Wang C, Jin Y, et al. Disocin prevents postmenopausal atherosclerosis in ovariectomized LDLR-/- mice through a PGC-1alpha/ERalpha pathway leading to promotion of autophagy and inhibition of oxidative stress, inflammation and apoptosis. *Pharmacol Res*. 2019;148:104414. doi:10.1016/j.phrs.2019.104414

82. Kwon CS, Sohn HY, Kim SH, et al. Anti-obesity effect of Dioscorea nipponica Makino with lipase-inhibitory activity in rodents. *Biosci Biotechnol Biochem*. 2003;67(7):1451–1456. doi:10.1271/bbb.67.1451
83. Wang P, He LY, Shen GD, Li RL, Yang JL. Inhibitory effects of Dioscin on atherosclerosis and foam cell formation in hyperlipidemia rats. *Inflammopharmacology*. 2017;25(6):633–642. doi:10.1007/s10787-017-0341-4
84. Wu SJ, Huang WC, Yu MC, et al. Tomatidine ameliorates obesity-induced nonalcoholic fatty liver disease in mice. *J Nutr Biochem*. 2021;91:108602. doi:10.1016/j.jnutbio.2021.108602
85. Li R, Liu Y, Shi J, et al. Diosgenin regulates cholesterol metabolism in hypercholesterolemic rats by inhibiting NPC1L1 and enhancing ABCG5 and ABCG8. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2019;1864(8):1124–1133. doi:10.1016/j.bbalip.2019.04.010
86. Navarro Del Hierro J, Casado-Hidalgo G, Reglero G, Martin D. The hydrolysis of saponin-rich extracts from fenugreek and quinoa improves their pancreatic lipase inhibitory activity and hypocholesterolemic effect. *Food Chem*. 2021;338:128113. doi:10.1016/j.foodchem.2020.128113
87. Elhanati S, Kanfi Y, Varvak A, et al. Multiple regulatory layers of SREBP1/2 by SIRT6. *Cell Rep*. 2013;4(5):905–912. doi:10.1016/j.celrep.2013.08.006
88. Li Y, Xu S, Mihaylova MM, et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab*. 2011;13(4):376–388. doi:10.1016/j.cmet.2011.03.009
89. Xu LN, Yin LH, Jin Y, et al. Effect and possible mechanisms of dioscin on ameliorating metabolic glycolipid metabolic disorder in type-2-diabetes. *Phytomedicine*. 2020;67:153139. doi:10.1016/j.phymed.2019.153139
90. Yao H, Tao X, Xu L, et al. Dioscin alleviates non-alcoholic fatty liver disease through adjusting lipid metabolism via SIRT1/AMPK signaling pathway. *Pharmacol Res*. 2018;131:51–60. doi:10.1016/j.phrs.2018.03.017
91. Liu M, Xu L, Yin L, et al. Potent effects of dioscin against obesity in mice. *Sci Rep*. 2015;5(1):7973. doi:10.1038/srep07973
92. Khateeb S, Albalawi A, Alkhedaide A. Regulatory effect of diosgenin on lipogenic genes expression in high-fat diet-induced obesity in mice. *Saudi J Biol Sci*. 2021;28(1):1026–1032. doi:10.1016/j.sjbs.2020.11.045
93. Fang K, Wu F, Chen G, et al. Diosgenin ameliorates palmitic acid-induced lipid accumulation via AMPK/ACC/CPT-1A and SREBP-1c/FAS signaling pathways in LO2 cells. *BMC Complement Altern Med*. 2019;19(1):255. doi:10.1186/s12906-019-2671-9
94. Uemura T, Goto T, Kang MS, et al. Diosgenin, the main aglycon of fenugreek, inhibits LXRalpha activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese diabetic mice. *J Nutr*. 2011;141(1):17–23. doi:10.3945/jn.110.125591
95. Poudel B, Lim SW, Ki HH, Nepali S, Lee YM, Kim DK. Dioscin inhibits adipogenesis through the AMPK/MAPK pathway in 3T3-L1 cells and modulates fat accumulation in obese mice. *Int J Mol Med*. 2014;34(5):1401–1408. doi:10.3892/ijmm.2014.1921
96. Ma W, Ding H, Gong X, et al. Methyl protodioscin increases ABCA1 expression and cholesterol efflux while inhibiting gene expressions for synthesis of cholesterol and triglycerides by suppressing SREBP transcription and microRNA 33a/b levels. *Atherosclerosis*. 2015;239(2):566–570. doi:10.1016/j.atherosclerosis.2015.02.034
97. Gai Y, Li Y, Xu Z, Chen J. Pseudoprotodioscin inhibits SREBPs and microRNA 33a/b levels and reduces the gene expression regarding the synthesis of cholesterol and triglycerides. *Fitoterapia*. 2019;139:104393. doi:10.1016/j.fitote.2019.104393
98. Sun B, Yin YZ, Xiao J. An In vivo estrogen deficiency mouse model for screening exogenous estrogen treatments of cardiovascular dysfunction after menopause. *J Vis Exp*. 2019;150:e59536.
99. Repa JJ, Liang G, Ou J, et al. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev*. 2000;14(22):2819–2830. doi:10.1101/gad.844900
100. Cheng S, Liang S, Liu Q, et al. Diosgenin prevents high-fat diet-induced rat non-alcoholic fatty liver disease through the AMPK and LXR signaling pathways. *Int J Mol Med*. 2018;41(2):1089–1095. doi:10.3892/ijmm.2017.3291
101. Moriwaki S, Murakami H, Takahashi N, et al. Yamogenin in fenugreek inhibits lipid accumulation through the suppression of gene expression in fatty acid synthesis in hepatocytes. *Biosci Biotechnol Biochem*. 2014;78(7):1231–1236. doi:10.1080/09168451.2014.915736
102. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res*. 2009;50 Suppl:S172–177. doi:10.1194/jlr.R800091-JLR200
103. Chang TY, Chang CC, Cheng D. Acyl-coenzyme A: cholesterolacyltransferase. *Annu Rev Biochem*. 1997;66:613–638. doi:10.1146/annurev.biochem.66.1.613
104. Heinonen TM. Acyl coenzyme A: cholesterolacyltransferase inhibition: potential atherosclerosis therapy or springboard for other discoveries? *Expert Opin Investig Drugs*. 2002;11(11):1519–1527. doi:10.1517/13543784.11.11.1519
105. Fujiwara Y, Kiyota N, Tsurushima K, et al. Tomatidine, a tomato sapogenol, ameliorates hyperlipidemia and atherosclerosis in apoE-deficient mice by inhibiting acyl-CoA: cholesterolacyl-transferase (ACAT). *J Agric Food Chem*. 2012;60(10):2472–2479. doi:10.1021/jf204197r
106. McKoy ML, Thomas PG, Asemota H, Omoruyi F, Simon O. Effects of Jamaican bitter yam (Dioscorea polygonoides) and diosgenin on blood and fecal cholesterol in rats. *J Med Food*. 2014;17(11):1183–1188. doi:10.1089/jmf.2013.0140
107. Temel RE, Brown JM, Ma Y, et al. Diosgenin stimulation of fecal cholesterol excretion in mice is not NPC1L1 dependent. *J Lipid Res*. 2009;50(5):915–923. doi:10.1194/jlr.M800631-JLR200
108. Hao S, Xu R, Li D, Zhu Z, Wang T, Liu K. Attenuation of streptozotocin-induced lipid profile anomalies in the heart, brain, and mRNA expression of HMG-CoA reductase by diosgenin in rats. *Cell Biochem Biophys*. 2015;72(3):741–749. doi:10.1007/s12013-015-0525-8
109. Marin-Medina A, Ruiz-Hidalgo G, Ble-Castillo JL, et al. Combined effect of diosgenin along with ezetimibe or atorvastatin on the fate of labelled bile acid and cholesterol in hypercholesterolemic rats. *Int J Environ Res Public Health*. 2019;16(4):627. doi:10.3390/ijerph16040627
110. Pirillo A, Norata GD, Catapano AL. LOX-1, OxLDL, and atherosclerosis. *Mediators Inflamm*. 2013;2013:152786. doi:10.1155/2013/152786
111. Gautier EL, Huby T, Witztum JL, et al. Macrophage apoptosis exerts divergent effects on atherogenesis as a function of lesion stage. *Circulation*. 2009;119(13):1795–1804. doi:10.1161/CIRCULATIONAHA.108.806158
112. Levitan I, Volkov S, Subbaiah PV. Oxidized LDL: diversity, patterns of recognition, and pathophysiology. *Antioxid Redox Signal*. 2010;13(1):39–75. doi:10.1089/ars.2009.2733
113. Li Y, Li Y, Yang T, Wang M. Dioscin attenuates oxLDL uptake and the inflammatory reaction of dendritic cells under high glucose conditions by blocking p38 MAPK. *Mol Med Rep*. 2020;21(1):304–310. doi:10.3892/mmr.2019.10806
114. Wang N, Tall AR. Regulation and mechanisms of ATP-binding cassette transporter A1-mediated cellular cholesterol efflux. *Arterioscler Thromb Vasc Biol*. 2003;23(7):1178–1184. doi:10.1161/01.ATV.0000075912.83860.26

115. Rosenson RS, Brewer HB Jr., Davidson WS, et al. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation*. 2012;125(15):1905–1919. doi:10.1161/CIRCULATIONAHA.111.066589
116. Lv YC, Yang J, Yao F, et al. Diosgenin inhibits atherosclerosis via suppressing the MiR-19b-induced downregulation of ATP-binding cassette transporter A1. *Atherosclerosis*. 2015;240(1):80–89. doi:10.1016/j.atherosclerosis.2015.02.044
117. Yu XH, Zhang DW, Zheng XL, Tang CK. Cholesterol transport system: an integrated cholesterol transport model involved in atherosclerosis. *Prog Lipid Res*. 2019;73:65–91. doi:10.1016/j.plipres.2018.12.002
118. Talbot CPJ, Plat J, Ritsch A, Mensink RP. Determinants of cholesterol efflux capacity in humans. *Prog Lipid Res*. 2018;69:21–32. doi:10.1016/j.plipres.2017.12.001
119. Kusano Y, Tsujihara N, Masui H, Shibata T, Uchida K, Takeuchi W. Diosgenin supplementation prevents lipid accumulation and induces skeletal muscle-fiber hypertrophy in rats. *J Nutr Sci Vitaminol (Tokyo)*. 2019;65(5):421–429. doi:10.3177/jnsv.65.421
120. Betters JL, Yu L. NPC1L1 and cholesterol transport. *FEBS Lett*. 2010;584(13):2740–2747. doi:10.1016/j.febslet.2010.03.030
121. Yu L, Li-Hawkins J, Hammer RE, et al. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest*. 2002;110(5):671–680. doi:10.1172/JCI0216001
122. Accatino L, Pizarro M, Solis N, Koenig CS. Effects of diosgenin, a plant-derived steroid, on bile secretion and hepatocellular cholestasis induced by estrogens in the rat. *Hepatology*. 1998;28(1):129–140. doi:10.1002/hep.510280118
123. Kosters A, Frijters RJ, Schaap FG, et al. Relation between hepatic expression of ATP-binding cassette transporters G5 and G8 and biliary cholesterol secretion in mice. *J Hepatol*. 2003;38(6):710–716. doi:10.1016/S0168-8278(03)00093-X
124. Nibbering CP, Groen AK, Ottenhoff R, Brouwers JF, vanBerge-Henegouwen GP, van Erpecum KJ. Regulation of biliary cholesterol secretion is independent of hepatocyte canalicular membrane lipid composition: a study in the diosgenin-fed rat model. *J Hepatol*. 2001;35(2):164–169. doi:10.1016/S0168-8278(01)00125-8
125. Nervi F, Bronfman M, Allalon W, Depiereux E, Del Pozo R. Regulation of biliary cholesterol secretion in the rat. Role of hepatic cholesterol esterification. *J Clin Invest*. 1984;74(6):2226–2237. doi:10.1172/JCI111649
126. Yu L, Gupta S, Xu F, et al. Expression of ABCG5 and ABCG8 is required for regulation of biliary cholesterol secretion. *J Biol Chem*. 2005;280(10):8742–8747. doi:10.1074/jbc.M411080200
127. Kosters A, Frijters RJ, Kunne C, et al. Diosgenin-induced biliary cholesterol secretion in mice requires Abcg8. *Hepatology*. 2005;41(1):141–150. doi:10.1002/hep.20540
128. Kamisako T, Ogawa H. Regulation of biliary cholesterol secretion is associated with abcg5 and abcg8 expressions in the rats: effects of diosgenin and ethinyl estradiol. *Hepatol Res*. 2003;26(4):348–352. doi:10.1016/S1386-6346(03)00153-0
129. Yu L, Lu H, Yang X, et al. Diosgenin alleviates hypercholesterolemia via SRB1/CES-1/CYP7A1/FXR pathway in high-fat diet-fed rats. *Toxicol Appl Pharmacol*. 2021;412:115388. doi:10.1016/j.taap.2020.115388
130. Spagnoli LG, Bonanno E, Sangiorgi G, Mauriello A. Role of inflammation in atherosclerosis. *J Nucl Med*. 2007;48(11):1800–1815. doi:10.2967/jnumed.107.038661
131. Peled M, Fisher EA. Dynamic aspects of macrophage polarization during atherosclerosis progression and regression. *Front Immunol*. 2014;5:579. doi:10.3389/fimmu.2014.00579
132. Devaraj S, Jialal I. C-reactive protein polarizes human macrophages to an M1 phenotype and inhibits transformation to the M2 phenotype. *Arterioscler Thromb Vasc Biol*. 2011;31(6):1397–1402. doi:10.1161/ATVBAHA.111.225508
133. Stoger JL, Gijbels MJ, van der Velden S, et al. Distribution of macrophage polarization markers in human atherosclerosis. *Atherosclerosis*. 2012;225(2):461–468. doi:10.1016/j.atherosclerosis.2012.09.013
134. Galic S, Fullerton MD, Schertzer JD, et al. Hematopoietic AMPK beta1 reduces mouse adipose tissue macrophage inflammation and insulin resistance in obesity. *J Clin Invest*. 2011;121(12):4903–4915. doi:10.1172/JCI58577
135. Binesh A, Devaraj SN, Devaraj H. Inhibition of nuclear translocation of notch intracellular domain (NICD) by diosgenin prevented atherosclerotic disease progression. *Biochimie*. 2018;148:63–71. doi:10.1016/j.biochi.2018.02.011
136. Zhao X, Yin L, Fang L, et al. Protective effects of dioscin against systemic inflammatory response syndrome via adjusting TLR2/MyD88/NFkappab signal pathway. *Int Immunopharmacol*. 2018;65:458–469. doi:10.1016/j.intimp.2018.10.036
137. Jung DH, Park HJ, Byun HE, et al. Diosgenin inhibits macrophage-derived inflammatory mediators through downregulation of CK2, JNK, NF-kappaB and AP-1 activation. *Int Immunopharmacol*. 2010;10(9):1047–1054. doi:10.1016/j.intimp.2010.06.004
138. Hirai S, Uemura T, Mizoguchi N, et al. Diosgenin attenuates inflammatory changes in the interaction between adipocytes and macrophages. *Mol Nutr Food Res*. 2010;54(6):797–804. doi:10.1002/mnfr.200900208
139. Chiu FL, Lin JK. Tomatidine inhibits iNOS and COX-2 through suppression of NF-kappaB and JNK pathways in LPS-stimulated mouse macrophages. *FEBS Lett*. 2008;582(16):2407–2412. doi:10.1016/j.febslet.2008.05.049
140. Choi KW, Park HJ, Jung DH, et al. Inhibition of TNF-alpha-induced adhesion molecule expression by diosgenin in mouse vascular smooth muscle cells via downregulation of the MAPK, Akt and NF-kappaB signaling pathways. *Vascul Pharmacol*. 2010;53(5–6):273–280. doi:10.1016/j.vph.2010.09.007
141. Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420(6917):868–874. doi:10.1038/nature01323
142. Breitenstein A, Camici GG, Tanner FC. Tissue factor: beyond coagulation in the cardiovascular system. *Clin Sci (Lond)*. 2009;118(3):159–172. doi:10.1042/CS20080622
143. Yang HP, Yue L, Jiang WW, Liu Q, Kou JP, Yu BY. Diosgenin inhibits tumor necrosis factor-induced tissue factor activity and expression in THP-1 cells via down-regulation of the NF-kappaB, Akt, and MAPK signaling pathways. *Chin J Nat Med*. 2013;11(6):608–615. doi:10.3724/SP.J.1009.2013.00608
144. Rytting E, Lentz KA, Chen XQ, Qian F, Vakatesh S. Aqueous and cosolvent solubility data for drug-like organic compounds. *AAPS J*. 2005;7(1):E78–105. doi:10.1208/aapsj070110
145. Manda VK, Avula B, Ali Z, et al. Characterization of in vitro ADME properties of diosgenin and dioscin from Dioscorea villosa. *Planta Med*. 2013;79(15):1421–1428. doi:10.1055/s-0033-1336521
146. Okawara M, Tokudome Y, Todo H, Sugibayashi K, Hashimoto F. Enhancement of diosgenin distribution in the skin by cyclodextrin complexation following oral administration. *Biol Pharm Bull*. 2013;36(1):36–40. doi:10.1248/bpb.b12-00467

147. Benghuzzi H, Tucci M, Eckie R, Hughes J. The effects of sustained delivery of diosgenin on the adrenal gland of female rats. *Biomed Sci Instrum*. 2003;39:335–340.
148. Okawara M, Hashimoto F, Todo H, Sugibayashi K, Tokudome Y. Effect of liquid crystals with cyclodextrin on the bioavailability of a poorly water-soluble compound, diosgenin, after its oral administration to rats. *Int J Pharm*. 2014;472(1–2):257–261. doi:10.1016/j.ijpharm.2014.06.032
149. Kim DH, Hong BN, Le HT, et al. Small molecular weight PEGylation of diosgenin in an in vivo animal study for diabetic auditory impairment treatment. *Bioorg Med Chem Lett*. 2012;22(14):4609–4612. doi:10.1016/j.bmcl.2012.05.094
150. Kesisoglou F, Panmai S, Wu Y. Nanosizing–oral formulation development and biopharmaceutical evaluation. *Adv Drug Deliv Rev*. 2007;59(7):631–644. doi:10.1016/j.addr.2007.05.003
151. Junghanns JU, Muller RH. Nanocrystal technology, drug delivery and clinical applications. *Int J Nanomedicine*. 2008;3(3):295–309. doi:10.2147/ijn.s595
152. Liu CZ, Chang JH, Zhang L, et al. Preparation and evaluation of diosgenin nanocrystals to improve oral bioavailability. *AAPS PharmSciTech*. 2017;18(6):2067–2076. doi:10.1208/s12249-016-0684-y
153. Pan CH, Tsai CH, Liu FC, et al. Influence of different particle processing on hypocholesterolemic and antiatherogenic activities of yam (Dioscorea pseudojaponica) in cholesterol-fed rabbit model. *J Sci Food Agric*. 2013;93(6):1278–1283. doi:10.1002/jsfa.5882
154. Cai B, Seong KJ, Bae SW, Chun C, Kim WJ, Jung JY. A synthetic diosgenin primary amine derivative attenuates LPS-stimulated inflammation via inhibition of NF-kappaB and JNK MAPK signaling in microglial BV2 cells. *Int Immunopharmacol*. 2018;61:204–214. doi:10.1016/j.intimp.2018.05.021
155. Parama D, Boruah M, Yachna K, et al. Diosgenin, a steroidal saponin, and its analogs: effective therapies against different chronic diseases. *Life Sci*. 2020;260:118182. doi:10.1016/j.lfs.2020.118182
156. Romero-Hernandez LL, Merino-Montiel P, Montiel-Smith S, et al. Diosgenin-based thio(seleno)ureas and triazolyl glycoconjugates as hybrid drugs. Antioxidant and antiproliferative profile. *Eur J Med Chem*. 2015;99:67–81. doi:10.1016/j.ejmech.2015.05.018
157. Tao X, Yin L, Xu L, Peng J. Dioscin: a diverse acting natural compound with therapeutic potential in metabolic diseases, cancer, inflammation and infections. *Pharmacol Res*. 2018;137:259–269. doi:10.1016/j.phrs.2018.09.022
158. Fox KM, Tai MH, Kostev K, Hatz M, Qian Y, Laufs U. Treatment patterns and low-density lipoprotein cholesterol (LDL-C) goal attainment among patients receiving high- or moderate-intensity statins. *Clin Res Cardiol*. 2018;107(5):380–388. doi:10.1007/s00392-017-1193-z
159. Komesaroff PA, Black CV, Cable V, Sudhir K. Effects of wild yam extract on menopausal symptoms, lipids and sex hormones in healthy menopausal women. *Climacteric*. 2001;4(2):144–150. doi:10.1080/cmt.4.2.144.150
160. Geberemeskel GA, Debebe YG, Nguse NA. Antidiabetic effect of fenugreek seed powder solution (Trigonella foenum-graecum L.) on hyperlipidemia in diabetic patients. *J Diabetes Res*. 2019;2019:8507453. doi:10.1155/2019/8507453
161. Shamshad Begum S, Jayalakshmi HK, Vidyavathi HG, et al. A novel extract of fenugreek husk (FenuSMART) alleviates postmenopausal symptoms and helps to establish the hormonal balance: a randomized, double-blind, placebo-controlled study. *Phytother Res*. 2016;30(11):1775–1784. doi:10.1002/ptr.5680
162. Wang ZL, Shi B, Liu ZJ. Effects of di'ao xinxuekang soft capsule on lipid peroxidation and the endothelial function in patients with coronary heart disease. *Chin J Integr Tradit West Med*. 2012;32(6):782–784. Chinese.
163. Al-Habori M, Raman A, Lawrence MJ, Skett P. In vitro effect of fenugreek extracts on intestinal sodium-dependent glucose uptake and hepatic glycogen phosphorylase A. *Int J Exp Diabetes Res*. 2001;2(2):91–99. doi:10.1155/EDR.2001.91
164. Cai B, Zhang Y, Wang Z, et al. Therapeutic potential of diosgenin and its major derivatives against neurological diseases: recent advances. *Oxid Med Cell Longev*. 2020;2020:3153082. doi:10.1155/2020/3153082
165. Aumsuwan P, Khan SI, Khan IA, et al. The anticancer potential of steroidal saponin, dioscin, isolated from wild yam (Dioscorea villosa) root extract in invasive human breast cancer cell line MDA-MB-231 in vitro. *Arch Biochem Biophys*. 2016;591:98–110. doi:10.1016/j.abb.2015.12.001
166. Li J, Liang Q, Li C, Liu M, Zhang Y. Comparative transcriptome analysis identifies putative genes involved in dioscin biosynthesis in Dioscorea zingiberensis. *Molecules*. 2018;23(2):454.
167. Sun W, Tu G, Zhang Y. A new steroidal saponin from Dioscorea zingiberensis Wright. *Nat Prod Res*. 2003;17(4):287–292. doi:10.1080/1478641031000136997
168. Cho J, Choi H, Lee J, Kim MS, Sohn HY, Lee DG. The antifungal activity and membrane-disruptive action of dioscin extracted from Dioscorea nipponica. *Biochim Biophys Acta*. 2013;1828(3):1153–1158. doi:10.1016/j.bbamem.2012.12.010
169. Kawabata T, Cui MY, Hasegawa T, Takano F, Ohta T. Anti-inflammatory and anti-melanogenic steroidal saponin glycosides from Fenugreek (Trigonella foenum-graecum L.) seeds. *Planta Med*. 2011;77(7):705–710. doi:10.1055/s-0030-1250477
170. Dong M, Feng XZ, Wu LJ, Wang BX, Ikejima T. Two new steroidal saponins from the rhizomes of Dioscorea panthaica and their cytotoxic activity. *Planta Med*. 2001;67(9):853–857. doi:10.1055/s-2001-18856
171. Oyama M, Tokiwano T, Kawaii S, et al. Protodioscin, isolated from the rhizome of dioscorea tokoro collected in northern japan is the major antiproliferative compound to HL-60 leukemic cells. *Curr Bioact Compd*. 2017;13(2):170–174. doi:10.2174/1573407213666170113123428
172. Wang T, Choi RC, Li J, et al. Antihyperlipidemic effect of protodioscin, an active ingredient isolated from the rhizomes of Dioscorea nipponica. *Planta Med*. 2010;76(15):1642–1646. doi:10.1055/s-0030-1249960
173. Hibasami H, Moteki H, Ishikawa K, et al. Protodioscin isolated from fenugreek (Trigonella foenumgraecum L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *Int J Mol Med*. 2003;11(1):23–26.
174. Gauthaman K, Adaikan PG, Prasad RN. Aphrodisiac properties of Tribulus Terrestris extract (Protodioscin) in normal and castrated rats. *Life Sci*. 2002;71(12):1385–1396. doi:10.1016/S0024-3205(02)01858-1
175. Tseng SC, Shen TS, Wu CC, et al. Methyl protodioscin induces apoptosis in human osteosarcoma cells by caspase-dependent and MAPK signaling pathways. *J Agric Food Chem*. 2017;65(13):2670–2676. doi:10.1021/acs.jafc.6b04800
176. Sun Z, Huang X, Kong L. A new steroidal saponin from the dried stems of Asparagus officinalis L. *Fitoterapia*. 2010;81(3):210–213. doi:10.1016/j.fitote.2009.09.002

177. Taylor WG, Zulyniak HJ, Richards KW, Acharya SN, Bittman S, Elder JL. Variation in diosgenin levels among 10 accessions of fenugreek seeds produced in western Canada. *J Agric Food Chem*. 2002;50(21):5994–5997. doi:10.1021/jf020486y
178. Friedman M. Tomato glycoalkaloids: role in the plant and in the diet. *J Agric Food Chem*. 2002;50(21):5751–5780. doi:10.1021/jf020560c