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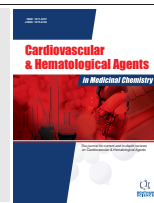
# The Cardiovascular Protective Effects of Chrysin: A Narrative Review on Experimental Researches

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**Abstract:** Chrysin is one of the flavonoids fruits, vegetables, and plant especially found in honey, it has been indicated that its cardiovascular protective effect is due to its antioxidative effects and anti-inflammatory activities. Chrysin exerts an antioxidant effect by enhancing the antioxidant system, suppressing pro-oxidant enzymes, scavenging free radicals and chelating redox active transition-metal ions. Chrysin decreases lipid synthesis and also increases its metabolism, thereby ameliorating blood lipid profile. Chrysin modulates vascular function by increasing the bioavailability of endothelial nitric oxide. Chrysin inhibits the development of atherosclerosis by decreasing vascular inflammation. The anti-inflammatory effects of chrysin may relate to its inhibitory effect on the nuclear transcriptional factor-kB signaling pathway. It also prevents vascular smooth muscle cells proliferation and thrombogenesis. Altogether, chrysin may be effective as a natural agent for the prevention and treatment of cardiovascular diseases; however, several clinical trial studies should be done to confirm its protective effects on humans.

**Keywords:** Antioxidant, apoptosis, cardiovascular disease, chrysin, inflammation, oxidative stress.

## 1. INTRODUCTION

Chrysin (5,7-dihydroxyflavone or 5,7-dihydroxy-2-phe-nyl-4Hchromen-4-one) belongs to the flavone class of the ubiquitous 15- carbon skeleton natural polyphenolic compounds which called flavonoids. The characteristic feature of flavones in chrysin is the presence C2-C3 double bond in ring C and the lack of oxygenation at C-3 (Fig. **1**). Unlike several flavonoids that have either one (most commonly at C-40) or two hydroxy (C30, C40 -diortho hydroxyl) group in ring-B, chrysin lacks oxygenation in this ring. Other natural derivatives of chrysin arise due to diversity in ring-A oxygenation including wogonin, oroxylin A and baicalein. Chrysin is found in various fruits, vegetables, and many plant extracts especially in honey, blue passion flower (Passifloracaerulea), and Propolis [1, 2]. The chemical structure of chrysin consists of three rings; two benzene rings (A and B) and an oxygen-containing ring (C), fused to the A ring (Fig. **1**) [3, 4].

Due to B and C-ring lack of oxygenation, the chemical properties of chrysin are associated with a number of pharmacological activities that range from antioxidant to anti-cancer effects [5]. However, variety in the chemical structure of flavones has shown that it can affect its antioxidant
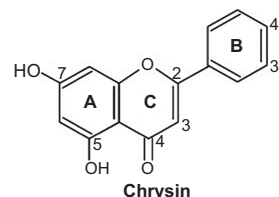


**Fig. (1).** Chemical structure of chrysin.

activity as well as the inhibitory effect on proinflammatory enzyme cyclooxygenase-2 (Cox-2) expression. For instance, luteolin, a main flavone, demonstrated greater Cox-2 inhibition than chrysin [6]. This has been related to chrysin's lack of 30, 40 hydroxylation on the "B" ring. Ko *et al.* showed that hydroxylation of 20 or 40 B rings was necessary for the inhibition of phorbol ester-induced Cox-2 expression by flavanones [7]. Hou *et al.* also demonstrated that an ortho-hydroxyl group is a requirement for the inhibition of LPS-induced Cox-2 expression in RAW 264.7 cells by anthocya-nins [8]. The presence of 30, 40 hydroxylations, a double bond between carbons 2 and 3 as well as a carbonyl group on carbon 4 have been reported to be essential to produce anti-oxidant activity [9]. After oral consumption by a human, the bioavailability of chrysin is low due to poor intestinal absorption, rapid metabolism, and exertion. The scientific evidence indicated that absorbed chrysin is catalyzed mainly by glucuronidation and sulfation [10]. Small amounts of chrysin are found as conjugates in the plasma and urine.

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Thus, fecal excretion may be the main route for chrysin exertion [11]. Lower doses of the flavonoids have been found safe for human use; however, the toxic effects of high doses of them have been reported. The allowable doses of chrysin for human consumption are 0.5 to 3 g every day. However, it was reported that chrysin (25 μM) induced liver toxicity in rainbow trout cell line [11]. It was also indicated that chrysin treatment induced DNA damage and cell death.

Although chrysin has low bioactivity, however, the amount of absorbed chrysin may be enough for protecting the cardiovascular diseases [12]. Many polyphenols have been shown to have potent and specific health-promoting activities when evaluated by high-performance *in vitro* assays as well as applied to animal models by injection. On the other hand, some of the polyphenols have been shown to have any therapeutical properties in man or animals when orally used. It seems, these effects induce through the poor bioavailability indicated by many polyphenols following the ingestion. The polyphenols similar to the most drugs, are regarded as xenobiotics by the body and must overcome many barriers, including chemical modification and extensive enzymatic activities during absorption and digestion, to reach their site(s) of function. This is especially real for polyphenols targeting the brain, that is supported by the firmly regulated blood-brain barrier. Surprisingly, several polyphenols are also identified to specially change many of the transport and metabolic phenomenon that control bioavailability. Therefore, there is an opportunity for increasing the bioactivity of polyphenols by controlling specific synergistic interactions with polyphenols that ameliorate their oral bioavailability. This idea should be discussed in future on several endogenous systems that prevent the bioavailability of ingested polyphenols to the brain, and our body. Therefore, the bioavailability may be ameliorated by especially controlling synergies between the orally used polyphenols.

Chrysin has been concentrated on its therapeutic properties in recent years [13-15]. Chrysin has been shown to be a very active flavonoid including many pharmacological properties such as antihypercholesterolemic activity [16], cardioprotective activity by improving post-ischemic functional recovery [17], suppressive effect on Vascular Endothelial Growth Factor (VEGF)-induced angiogenesis [18], anti-inflammatory activity by blocking histamine release and proinflammatory cytokine expression [19]. In addition to all these pharmacological properties of chrysin, it has also been indicated to have a neuroprotective activity acting through various mechanisms. However, unlike other flavonoids, the therapeutic properties of chrysin remain nascent in current literature due to issues with absorption and bioavailability. There is also numerous scientific literature that indicates the cardioprotective effects of chrysin [11-15]. According to biomedical findings, chrysin has antioxidant, anti-inflammatory, anti-atherogenic, anti-hypertensive and anti-diabetic effects [16-20]. The cardioprotective effect of chrysin was strongly confirmed by experimental studies [21, 22]. Thus, the present study has been designed to review the current literature on chrysin and cardiovascular health with the main attention on studies which involved in the cardioprotective effect and its underlying mechanisms.

## 2. CHRYSIN AND CARDIOVASCULAR SYSTEM

Several mechanisms are responsible for the progression of CVDs including oxidative stress, inflammation, dyslipidemia, vascular endothelial cell dysfunction, platelet aggregation, and the proliferation of vascular cells [22]. Chrysin exerts its cardioprotective effects by modulating some cellular signaling pathways that induce inflammation, oxidative, nitrosative stress, apoptosis, platelet aggregation, and vascular cells dysfunction [22]. The cardiovascular pathway targets affected by chrysin have been discussed below.

## 3. THE ANTIOXIDANT EFFECTS OF CHRYSIN AND CARDIOVASCULAR HEALTH

### 3.1. Oxidative Stress and CVDs

Oxidative stress plays a main role in the development of various CVDs such as atherosclerosis, hypertension, ischemic heart disease, cardiac hypertrophy, cardiomyopathies and congestive heart failure [23-27]. The Reactive Oxygen Species (ROS) at normal levels act as signaling molecules to modulate the cardiovascular system and preserve its homeostasis [28]. In the CVDs, ROS are generated in the mitochondria by NADPH oxidases (NOX), oxidases (LO), Xanthine Oxidases (XO), and myeloperoxidases (MPO). There is a close link between mitochondrial-ROS (mtROS) production and endothelial dysfunction. The endothelial dysfunction is caused by mtROS and also $^-O_2$ generation is increased in damaged endothelial cells. In the endothelial cells, NO is necessary to protect its normal function [29-32].

### 3.2. Chrysin as an Antioxidant Protects CVDs

Several studies have indicated that natural antioxidants can improve CVDs by reducing oxidative stress [33-35]. In this context, the antioxidant properties of chrysin and its effects on cardiovascular problems have been investigated [36, 37]. The direct and indirect antioxidant effects of chrysin on cardiovascular tissue have been demonstrated [38, 39]. The antioxidant effect of chrysin is mostly due to its redox activities, donating an electron/hydrogen atom, quenching singlet oxygen molecule and its metal chelating potential [40]. The antioxidant effects of chrysin are related to the presence of hydroxyl groups in the $5^{th}$ and $7^{th}$ position of the aromatic rings [40]. Anandhi *et al.* (2013) indicated the protective effects of chrysin against Triton-induced hypercholesterolemia in rats. Chrysin modulated hepatic lipid metabolism by inhibiting oxidative stress [41].

### 3.3. Chrysin Protects Atherosclerosis

Atherosclerosis, the main type of CVDs, is determined by plaque formation in the inner walls of coronary arteries, containing LDL-c, cellular waste, and surrounding materials [41]. The initial stages of atherogenesis are caused by ox-LDL, which activates the endothelium by the generation of adhesion molecules, which recruit leukocytes to injured site and secrete pro-inflammatory cytokines and ROS. The ROS induce apoptosis, necrosis and, lead to thrombosis. As noted above, ROS have a close link with the hyperlipidemia, atherosclerosis and other CVDs [41].

The findings of the study indicated that oral administration of chrysin (200 mg/kg, for 7 days) reduced the hepatic levels of lipid peroxidation and also elevated the levels of antioxidants in the Triton WR-1339-induced hypercholesterolemic rats [41].

In addition, chrysin (200 mg/kg for 15 days, orally) reduced the hepatic levels of lipid peroxidation (malondialdehyde-MDA) and also elevated the levels of enzymatic (CAT, SOD, and GPx) and non-enzymatic (GSH) antioxidants in an experimental model of atherosclerosis [41]. Chrysin reversed the Atherogenic Index (AI) (AI= (TC − HDL) / HDL), the serum levels of LDL-c and Very Low-Density Lipoprotein (VLDL)-c to the normal levels in Triton-treated rats [41]. It was suggested that the increase in enzymatic and non-enzymatic antioxidants in the liver protected hepatic tissue from lipidemic-oxidative stress and prevented the progression of atherosclerosis in rats fed with an atherogenic and hypercholesterolemic diet.

### 3.4. Chrysin and Pulmonary Hypertension

The protective effects of chrysin against rat pulmonary vascular remodeling in hypoxia-induced pulmonary hypertension have been investigated. Pulmonary hypertension is caused by the proliferation and migration of Pulmonary Artery Smooth Muscle Cells (PASMCs) and accumulation of extracellular matrix (ECM) components, including collagens [42]. Oxidative stress is involved in the development of pulmonary hypertension. Chrysin (50 and 100 mg/kg for 4 weeks, SC) treatment significantly ameliorated pulmonary vascular remodeling by decreasing the expression of NADPH oxidase 4 (NOX4) in pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. In addition, chrysin decreased collagen I and III expressions and the levels of ROS and MDA induced by hypoxia in cultured PASMCs. ROS play a main role in the regulation of vascular tone and function [42]. Acute hypoxic vasoconstriction in the lung is accompanied by an increase in ROS production [42]. It was found that the induction of NOX4 contributes to systemic vascular pathology by increasing ROS production [42]. ROS lead to PASMC proliferation and pulmonary vascular modifications in animal models. The hemodynamic and histological findings of the study suggested that chrysin treatment ameliorated the increased NOX4 and collagen expressions *in vivo* and *in vitro* models. Chrysin also decreased PASMC proliferation, ROS and MDA production induced by hypoxia *in vitro* [43].

### 3.5. Chrysin and Hypertension

Chrysin also ameliorated hypertension in L-NAME-induced hypertensive rats by modulating oxidative stress in the aorta, heart, and blood [44]. The NO synthesis *via* chronic inhibition by NOS inhibitors, such as L-NAME, causes endothelial dysfunction and an elevation in blood pressure [44]. It was reported that L-NAME caused vasoconstriction and changed the renin-angiotensin system in animal models. Oxidative stress plays a main role in the pathogenesis of hypertension and its complications [44]. The study indicated that chrysin inhibited the membrane damage by decreasing the levels of lipid peroxidation and increasing the antioxidant

content in endothelial cells of L-NAME-induced hypertensive rats [44].

In addition, chrysin also decreased the oxidative stress by ameliorating the expression of iNOS. Endothelial-derived NO from activation of constitutive NO synthase is an essential factor for preserving vascular tone and homeostasis [40]. However, the over-production of NO following iNOS secretion of immune cells induces oxidative damage [40]. The reaction between NO and free radicals produces the highly corrosive OONO that causes oxidized-Low Density Lipoprotein (ox-LDL), leading to severe damage to the endothelial cells. Chrysin (50μM) prevented the expression of iNOS in LPS-activated cultured RAW 264.7 monocyte/macrophages by decreasing the activation of Nuclear transcriptional Factor-kB (NF-kB), thereby decreasing NO production [45]. Altogether, this section of the present review confirmed the ability of chrysin to prevent cardiovascular diseases by modulating oxidative stress. Fig. (**2**) indicates the association between the antioxidant effects of chrysin and its cardioprotective effects.

## 4. THE HYPOLIPIDEMIC EFFECTS OF CHRYSIN AND CARDIOVASCULAR HEALTH

Dyslipidemia is caused by the abnormalities in plasma lipids (cholesterol, triglycerides (TGs), LDL-c, High-Density Lipoprotein (HDL-c) that leads to the development and progression of CVDs. Chrysin can modulate lipid profile through different mechanisms and inhibit the progression of atherosclerotic plaque in animal models of hyperlipidemia [41]. Chrysin (200 mg/kg, PO, for 7 days) has been indicated to decrease the serum levels of cholesterol, TGs, LDL-c, and VLDL-c and also inhibit the cholesterol accumulation in the liver of rats that received a single intraperitoneal injection of Triton WR-1339 [41]. The study suggested that the hypolipidemic effects of chrysin may be related to its modulating effects of oxidative stress.

They also reported that chrysin (200 mg/kg, PO, for 15 days) decreased the serum levels of total cholesterol, TGs, LDL-c, and VLDL-c serum levels also increased the serum levels of HDL-c in rats fed with an atherogenic diet [46]. It was indicated that chrysin decreased the intestinal absorption of cholesterol by decreasing the incorporation of dietary and biliary cholesterol into micelles [46]. It has been suggested that chrysin has hypolipidemic effects by reducing lipid absorption. In addition, chrysin increased the activities of the LPL in liver tissue and HMG-CoA reductase in the rats fed with an atherogenic diet [46].

It was indicated that chrysin ameliorated oxidative stress in the liver of rat with atherogenic diet-fed. Oxidative stress plays an important role in the pathogenesis of hypercholesterolemic atherogenesis. The study suggested that chrysin protected animals against atherogenic diet-fed, *via* decreasing of serum lipid levels, modulating metabolic enzymes, and oxidative stress in the liver [47]. El-Bassossy *et al.*, 3013 indicated that chrysin (25 mg/kg, PO, for 6 weeks) improved dyslipidemia in streptozotocin (STZ) diabetic rats [47]. They indicated that the modulatory effect of chrysin on dyslipidemia is related to the stimulation of Peroxisome Proliferator-Activated (PPARγ) Receptors. PPARγ controls fatty acid storage and glucose metabolism [47]. The activated
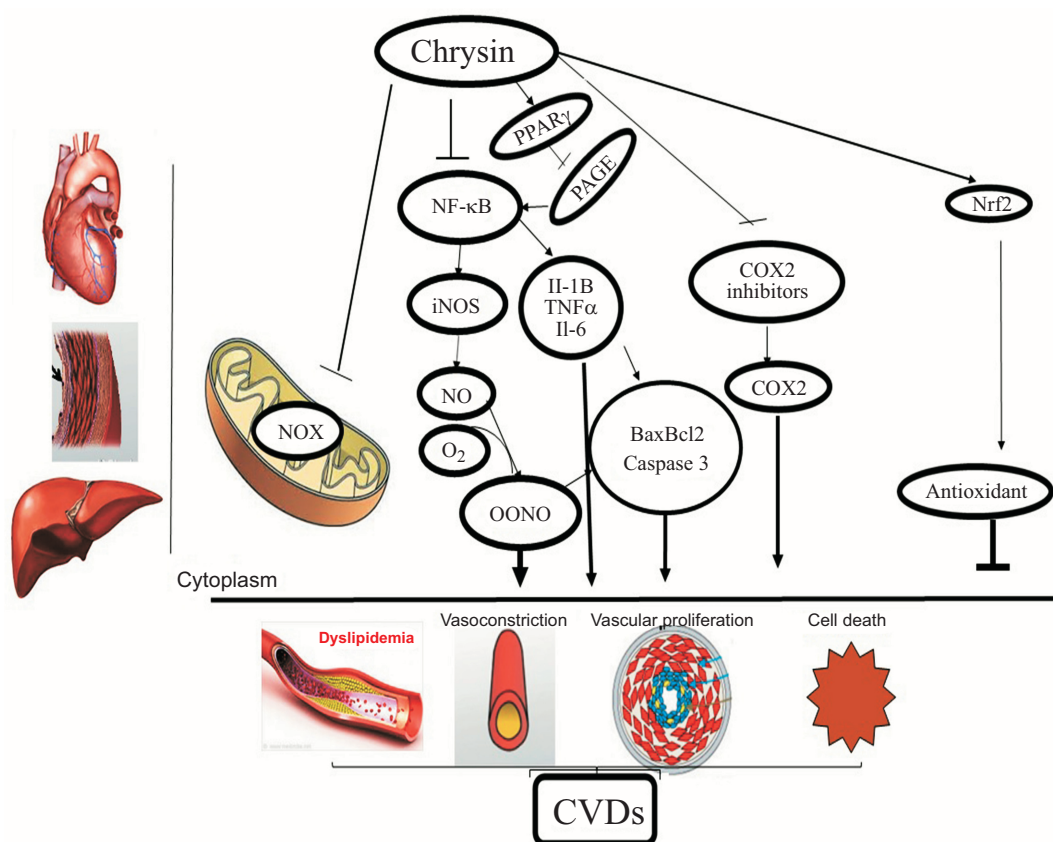
**Fig. (2).** Scheme summarizing the assocation between anti-oxidant, anti-inflammatory and anti-apoptotic efefcts of chrysin and its cardiovascular protective effects.

genes by PPARγinduce the lipid uptake and adipogenesis in fat cells [47]. Veerappan and Senthilkumar, 2015 indicated that chrysin (25 mg/kg, PO, for 4 weeks) decreased the Blood Pressure (BP) and lipid profile in L-NAME-induced hypertensive rats. Hypertension is usually accompanied by hyperlipidemia due to liver dysfunction following high BP [44]. The association between decreased NO and increased cholesterol levels seen in the study indicated that chrysin ameliorated dyslipidemia in the hypertensive model by increasing NO bioavailability and decreasing oxidative stress in the liver. Samarghandian *et al.* 2016 also indicated the hypolipidemic effects of chrysin in STZ-diabetic rats. They found that chrysin (20, 40 and 80 mg/kg, IP, for 4 weeks) modulated serum lipid profile in diabetic animals [48]. In addition, they observed that chrysin modulated the oxidative stress indices in the serum and liver of diabetic rats. It was suggested that the modulatory effect of chrysin on oxidative stress is one of the main mechanism for its hypolipidemic activity [48]. Ramírez-Espinosa *et al.*, 2016 also found that chrysin (50 mg/kg, PO, for 10 days) modulated the serum lipid profile in hyperglycemic athymic nude mice [49]. In chrysin-treated animals, lipid profile showed important alterations in LDL-c levels. The findings indicated that fatty acids metabolism is being shifted towards LDL-c production following PPAR-γ activation [49]. Fig. (**3**) indicates the association between hypo-lipidemic effects of chrysin and its cardioprotective effects.

## 5. THE VASCULAR HEMOSTATIC EFFECT OF CHRYSIN AND CARDIOVASCULAR HEALTH

Vascular endothelial cells as a biological barrier play a pivotal role in fluid filtration, blood vessel tone and vascular homeostasis [50]. Endothelial dysfunction is responsible for the initiation and progression of various CVDs [50]. Recently, it has been focused on the implication of natural and chemical drugs that inhibit ECs injuries or improve endothelial function [50]. In this context, one study indicated that chrysin ameliorated endothelial function in animal models of CVDs.

The findings indicated that chrysin could ameliorate vascular relaxation by activating cyclic guanosine monophosphate (cGMP) pathway. cGMP reduces $Ca^{2+}$ sensitivity of contractile components *via* activation of cGMP-kinase [51]. cGMP stimulates the protein kinase G, thereby modulates vascular smooth muscle cell relaxation. Furthermore, diabetes caused an increase in the levels of TGs, total and LDL-c, while chrysin ameliorated this dyslipidemia.

Chrysin also decreased the serum levels of Advanced Glycation End products (AGEs) level in diabetic animals. The study indicated that chrysin ameliorated diabetes-induced impairment in endothelial-dependent vaso-relaxation *via* ameliorating lipid profile and AGEs [51]. In addition, it was suggested that the vasculoprotective effect of chrysin may be related to PPARγ activation. However, the other study
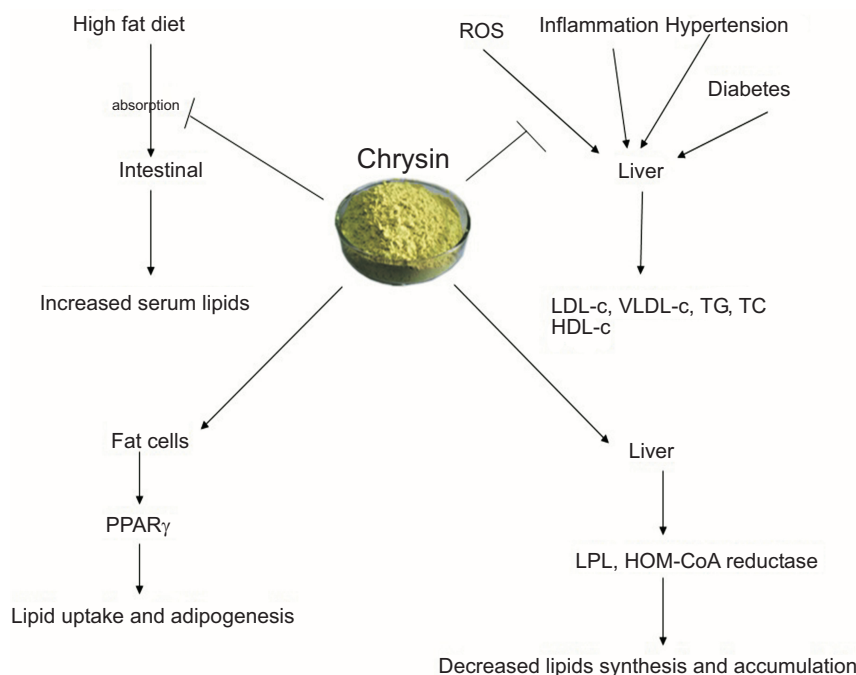
**Fig. (3).** Scheme summarizing the assocation between hypo-lipidemic effect of chrysin and its cardiovascular protective effects.
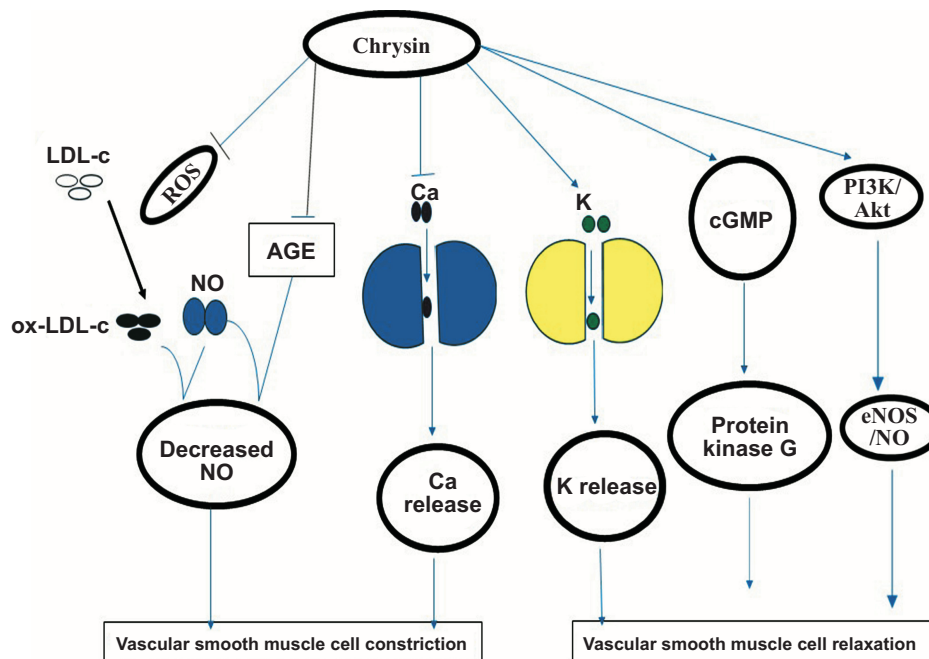


**Fig. (4).** Scheme summarizing the assocation between vascular hemostatic effect of chrysin and its cardiovascular protective effects.

indicated that chrysin (5 μM) increased the expression of eNOS in isolated rat aorta through PI3K/Akt and cAMP-dependent protein kinase A, proposing a genomic effect of this agent. The increased eNOS activity, endothelium-derived NO production and cyclic guanosine-3′,5′-mono-phosphate (cGMP) content in the arterial tissues of rats [52], propose that chrysin may regulate endothelium-dependent vasorelaxation *via* eNOS/NO signaling pathway [52, 53].

Chrysin also increased the NO bioavailability and ameliorated endothelial function by combating against ROS [54]. It was suggested that the relaxant activities of chrysin may be related to the release of NO and prostaglandins from the endothelium and also the inhibition of Ca$^{+2}$ influx and an increase of Ca$^{+2}$ efflux intracellularly [54]. Chrysin (1 μM-0.1 mM) has been indicated to increase isolated rat aorta vaso-relaxation responses to potassium in a dose-dependent manner [55]. The studies suggested that the endothelium-independent vasorelaxing activity of chrysin and also indicated ATP-sensitive potassium channels may be involved in its mechanism. Fig. (**4**) indicates the association between vascular hemostatic effects of chrysin and its cardioprotective effects [56].

## 6. ANTI-INFLAMMATORY EFFECTS OF CHRYSIN AND CARDIOVASCULAR HEALTH

Inflammation is associated with CVDs such as atherosclerosis and hypertension [57]. Epidemiological findings indicated that inflammation indices are significantly increased in a patient with cardiovascular diseases [30]. Recently, inflammation is considered a main therapeutic target for CVDs treatment [58]. It was reported that chrysin prevented cardiovascular diseases due to its anti-inflammatory effects [58]. Chrysin has been indicated to exhibit anti-inflammatory activity *via* several mechanisms [58]. The suppressive effect of chrysin on cyclooxygenase2 (COX-2) inhibitor is one of the mechanisms underlying anti-inflammatory activities of this compound [58]. COX-2 is one of the prostanoids that is secreted under certain inflammatory conditions and deteriorates the progression of inflammation in the body. COX-2 is produced in macrophages, leukocytes, and fibroblasts upon cytokines stimulation and involved in the prostaglandin synthesis [58].

It was indicated that chrysin (25, 50, or 100 µg/l) inhibited inflammation-related Cox-2 expression and PGE2 synthesis in RAW 264.7 macrophage-like cells exposed to LPS [59]. The modulation of NO production is the other mechanism by which chrysin inhibits the inflammation events. NO has a critical role in the homeostasis of vascular cells, both in normal and pathological condition [59]. The production of NO by iNOS may lead to inducing localized and generalized inflammatory responses after cell damage [59]. The activation of NF-κB has been indicated to increase the expression of iNOS. The inhibition of iNOS may be a main therapeutic purpose [58]. In this regards, the effect of chrysin on the expression of iNOS in cultured RAW 264.7 monocyte/macrophages stimulated with LPS and interferon-y (IFNy) has been investigated [59]. The findings indicated that chrysin (50 µM) significantly decreased the iNOS gene expression.

Although chrysin had no effect on the NF-κB expression, this compound markedly decelerated the DNA-binding activity of Interferon Regulatory Factor 1 (IRF-1), which regulated the synergistic effect of IFNy on iNOS gene. The study suggested that the inhibitory effect of chrysin on the IRF-1 activation indicated "a redox-sensitive step in the activation of this transcription factor, which in contrast to NF-KB requires de novo protein synthesis" [59]. However, Lee *et al.*, 2009 indicated that chrysin decreased TNF-α-induced inflammation in HEK 293 cells *via* inhibition of phosphorylation and degradation of IkB-α and translocation of NF-kB [60]. Li *et al.* 2015 also confirmed that chrysin (50 and 100 mg/kg) ameliorated the right ventricular remodeling in a rat model of monocrotaline-induced Pulmonary Arterial Hypertension (PAH) *via* inhibiting NOX4 and NF-κB expression [61]. Ramirez-Episonsa *et al.*, 2017 indicated that chrysin (50 mg/kg, for 10 days) ameliorated the diabetic effects of STZ in nude mice by decreasing inflammatory cytokines such as IL-1β and TNF-α [47].

During hyperglycemia, the activated NF-κB increased synthesis and secretion of TNF-α and IL-1β [62]. The study suggested that chrysin may prevent the progression of diabetes and its complications by suppressing the expression of NF-Kβ [63]. Binding of AGEs to a Receptor for AGE (RAGE) induces over-production of ROS, peroxynitrite formation, and recruitment of inflammatory cytokines [63]. Additionally, AGE-RAGE interaction induces NF-κB signaling pathway [3-6]. Chrysin (60 mg/kg, PO, for 28 days) as a PPAR-$\gamma$ agonist also ameliorated the isoproterenol-induced myocardial injury in STZ-diabetic rats. The findings indicated that chrysin improved myocardial injury in diabetic rats by increasing PPAR-$\gamma$ expression and decreasing RAGE expression [64]. Chrysin reduced inflammation *via* inhibiting NF-$\kappa$ Bp65/IKK-$\beta$ expression and TNF-$\alpha$ level [64]. Chrysin decreased apoptosis through increasing Bcl-2 expression and decreasing Bax and caspase-3 expressions [64]. Additionally, chrysin decreased nitro-oxidative stress by ameliorating the expressions of in TBARS, 8-OHdG, NO, NT, eNOS, Nox4, GSH, CAT and SOD [64]. The study suggested that chrysin ameliorated cardiovascular diseases by inhibiting AGE-RAGE interaction [64].

Fig. (**2**) indicates the association between the anti-inflammatory effects of chrysin and its cardioprotective effects.

## 7. ANTI-PROLIFERATIVE EFFECTS OF CHRYSIN AND CARDIOVASCULAR HEALTH

Vascular Smooth Muscle Cells (VSMCs) proliferation and migration usually occur during vascular development or repair [65]. However, pathological proliferation and migration are the major events in vascular remodeling and atherosclerotic plaque formation [65]. Vascular damage causes oxidative stress and increased generation of ROS in the vessel wall [65]. Additionally, ROS are generated and play as molecular signaling for the induction of Receptor Tyrosine Kinases (RTKs) during vascular damage. Platelet-derived growth factor (PDGF) activation acts as a key role in VSMC proliferation and migration. PDGF receptors and ligands are markedly increased in atherosclerotic plaques [65]. PDGF-receptors and other tyrosine kinases contribute to VSMC proliferation and migration are modulated by Protein Tyrosine Phosphatases (PTPs) [66]. It was indicated that chrysin prevented the proliferation of VSMCs induced by PDGF [67]. However, how chrysin inhibits VSMC proliferation is not fully understood. Chrysin treatment has been indicated to prevent PDGF-induced proliferation and chemotaxis and decreased PDGF signaling in VSMCs [67]. This effect of chrysin on VSMC proliferation may be related to inhibition of PDGF activity that induced ROS production and NADPH oxidase activation [67].

Chrysin enhanced the Protein Tyrosine Phosphatase (PTP) activity that prevented the function of PDGF receptors [67]. The effect of chrysin on PDGF signaling and PTP activity was deteriorated by the reduction of glutathione (GSH), indicating the effect of chrysin on GSH content for PTP reactivation [67]. The study suggested that chrysin modulation prevented PDGF-induced VSMC proliferation and neointima formation in the rat artery by modulating intracellular PTP activity [67]. In addition, it was reported that chrysin prevented pulmonary vascular remodeling in hypoxia induced-pulmonary hypertension in rats [43]. The study indicated that chrysin (50 and 100 mg/kg, SC, for 4 weeks) decreased the expression of NADPH oxidase (NOX4) and also collagen I and III in a rat model of pulmonary hyperten-

sion. In addition, chrysin (1, 10 and 100 μM) inhibited NOX4, collagen I and III, ROS and MDA generation, as well as PVSMCs proliferation in cultured PVSMCs [43]. The findings suggested that chrysin inhibited PVSMCs proliferation by decreasing NOX4 expression and also ROS and MDA generation [43]. Fig. (**5**) indicates the association between the anti-proliferative effects of chrysin and its cardioprotective effects.
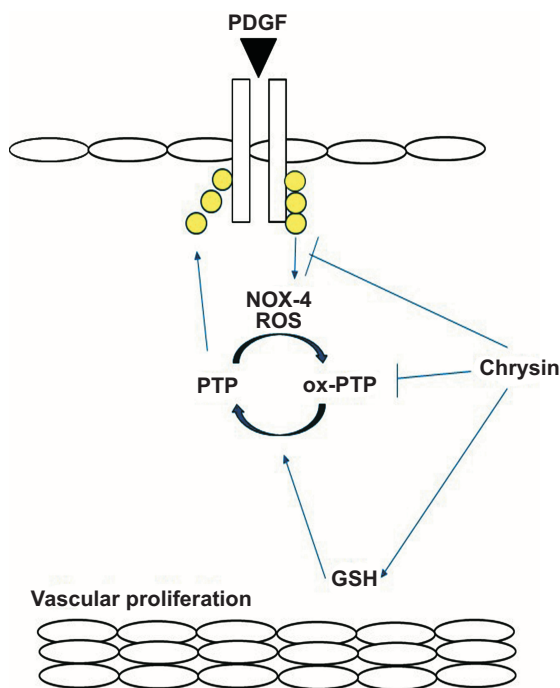


**Fig. (5).** Scheme summarizing the assocation between antiproliferative effect of chrysin and its cardiovascular protective effects.

## 8. ANTI-APOPTOTIC EFFECTS OF CHRYSIN AND CARDIOVASCULAR HEALTH

Apoptosis of cardiac muscle cells has been recognized as the main factor in the progression of heart failure. There are several apoptosis-based therapeutic strategies to prevent heart failure [68]. In this regards, Angel *et al.* 2013 indicated that chrysin (50 mg/kg) decreased cardiomyocyte apoptosis and loss of intermediate filaments in a mouse model of mitoxantrone cardiotoxicity [69]. The findings indicated that chrysin decreased serum levels of Creatine Kinase (CK), the expression of Bax and caspase-3 and also increased Bcl-2 and desmin expressions in the cardiomyocyte of mitoxantrone-treated mice [69]. The study suggested that chrysin prevented mitoxantrone-induced cardiomyocyte apoptosis by reducing the *Bax/Bcl-2* ratio and caspase-3 expression [69]. Chrysin (60 mg/kg for 28 days, orally) also reduced Bax and caspase-3 expression and increased *Bcl-2* expression in the myocardium of streptozotocin-diabetic rats exposed to isoproterenol [69].

In addition, GW9662 (PPARγ antagonists) prevented the anti-apoptotic effect of chrysin, therewith it was suggested that PPAR-γ has the main role in the anti-apoptotic activity of chrysin. Activation of PPAR-γ inhibits the AGE/RAGE-induced apoptosis, which may be one of the underlying

mechanisms of anti-apoptotic activity of chrysin. The findings suggested that the anti-apoptotic effects of chrysin may be related to PPAR-γ activation, which ameliorated AGE/RAGE-mediated apoptotic responses [69].

## 9. ANTI-PLATELET EFFECTS OF CHRYSIN AND CARDIOVASCULAR HEALTH

Presently, CVDs are considered as one of the main causes of mortality worldwide, that thrombosis plays the main role in the rate of mortality due to this disease [70]. Activated platelet is the major risk factor for the plaque rupture and thrombosis disease [71]. Following vessel wall injury, collagen binds to the platelet *via* VWF receptor that activated the glycoprotein VI (GPVI)/FcRγ signaling pathway, including PLCγ2, and PI3K. This pathway causes cytoplasmic calcium mobilization, platelet shape alteration, and granule release [71]. Activated platelet secrets clotting mediators such as thromboxane A2 that enhance the activation and recruiting of platelet, leading to the progression thrombus [71]. Integrin αIIbβ3 is a receptor on the surface of activated platelets that binds to plasma fibrinogen, leading to platelets aggregation and coagulation [71].

MAP kinase is a serine/threonine kinase, including ERK1/2, JNK1/2, and p38 MAP kinase that induces the platelet activation, adhesion, and aggregation [71]. Platelet inhibition has been considered as a target for the treatment of CVDs [72]. In this context, it was reported that chrysin prevented platelet aggregation and granule release induced by collagen, and also platelet aggregation induced by ADP, U46619, and thrombin on collaged-activated platelets. Additionally, chrysin decreased the number of adherent platelet as well as inhibits collagen-induced activation of Syk, PKC, PLCγ2, and the phosphorylation of Akt and ERK1/2. Chrysin also increased the phosphorylation of molecules, including FcγRIIa, FAK, Akt, and GSK3β that prevented the platelet aggregation [73]. Ravishankar *et al.* 2017 also indicated that chrysin decreased "collagen-related peptide (CRP-XL)-induced platelet aggregation, inside-out signaling to integrin αIIbβ3, granule secretion and integrin αIIbβ3 mediated outside-in signaling in platelets". In addition, chrysin decreased thrombus formation in human blood cells under arterial flow conditions and extended the bleeding time in mice [74]. Fig. (**6**) indicates the association between the antiplatelet effect of chrysin and its cardioprotective effects.

## 10. TOXIC EFFECTS OF CHRYSIN

Chrysin at low doses which is present in our diet may be safe for our body. However, chrysin may totally not safe after exposing the high doses of these products that usually present in food supplements. Chrysin at a low dose can antagonize aromatase, therefore, inhibiting the conversion of testosterone to estradiol, a positive effect for the body for increasing the muscle mass. However, whether or not this is a real effect of chrysin in our body has been significantly challenged in different articles [75-77]. Although there are several articles that showed the therapeutical properties of chrysin. However, as shown in the different research, chrysin was the most toxic compound against the trout liver cells. It seems that the toxic effects of chrysin in the trout hepatocytes may involve the peroxidase-like activity in the trout
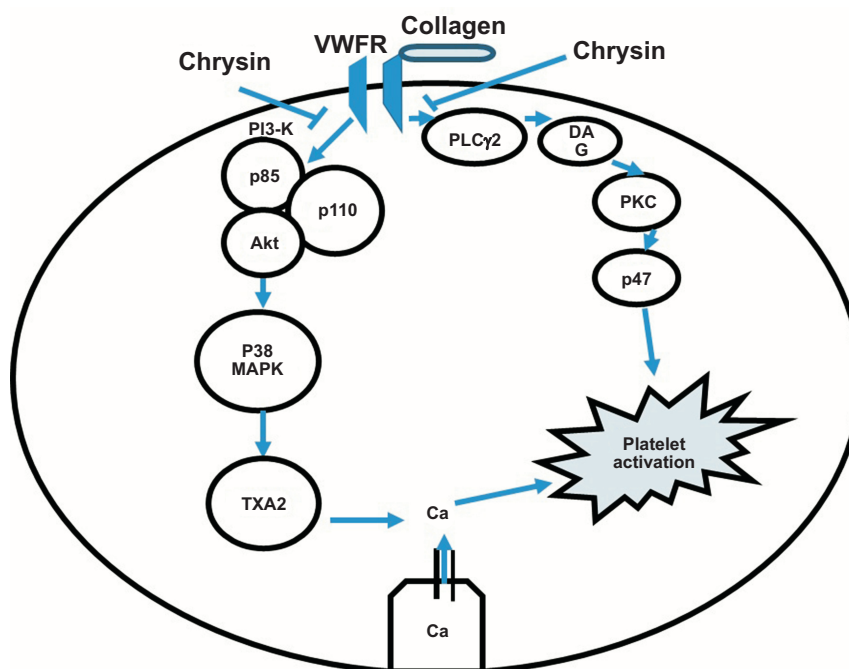
**Fig. (6).** Scheme summarizing the assocation between anti-plathelet effect of chrysin and its cardiovascular protective effects.

**Table 1.** Summary of cardiovascular protective effects of chrysin.

| Agent | Main Field of Activity | Mechanism | Refs. |
|---|---|---|---|
| Chrysin | Modulating oxidative stress | Decreased the hepatic levels of lipid peroxidation Increased the levels of enzymatic and non-enzymatic antioxidants | [41] |
| | | Decreased NOX expression and the levels of ROS and MDA | [43] |
| | | Decreased the levels of lipid peroxidation/Increased the levels of enzymatic and non-enzymatic antioxidants in aorta, heart, and blood | [44] |
| | | Decreasing the activation of NF-kB and NO production | [45] |
| | Modulating lipid synthesis and metabolism | Increased the activities of the LPL and HMG-CoA reductase in liver tissue | [47] |
| | | Increased expression of PPARg receptors | [44] |
| | | Increased NO bioavailability and decreased oxidative stress in liver | [48] |
| | | Increased LDL-c metabolism *via* inducing the activation of PPAR-γ | [49] |
| | Modulating vascular hemostasis | Ameliorated No production | [51] |
| | | Regulated endothelium-dependent vasorelaxation *via* eNOS/NO signaling pathway | 52] |
| | | Inhibited $Ca^{+2}$ influx and increased $Ca^{+2}$ efflux | [54] |
| | | Increased aorta vasorelaxation *via* activating of potassium channels | [55] |
| | Modulating inflammation | Decreased Cox-2 expression and PGE2 synthesis Decreased the iNOS generation *via* inhibiting the expression of NF-KB | [58, 59] |
| | | Inhibited phosphorylation and degradation of IkB-α and translocation of NF-kB | [60] |
| | | Prevented hypertension *via* inhibiting NOX4 and NF-kB expression | [61] |
| | | Decreased the levels of TNF-α and IL-1β *via* inhibiting NF-kB expression | [47] |
| | | Decreased inflammation *via* inhibiting NF-κBp65/IKK-β expression | [64] |

**(Table 1) contd….**

| Agent | Main Field of Activity | Mechanism | Refs. |
|-------|----------------------|-----------|-------|
| Chrysin | Modulating proliferation | Inhibited PDGF function-induced ROS production and NADPH oxidase activation | [67] |
| | | Inhibited PVSMCs proliferation by decreasing NOX4 expression and also ROS and MDA generation | [43] |
| | Modulating apoptosis | Decreased mitoxantrone-induced cardiomyocyte apoptosis by reducing of the Bax/Bcl-2 ratio and caspase-3 expression | [69] |
| | Modulating platelet activity | Inhibited the phosphorylation of Syk, PKC, PLCg2, Akt and ERK1/2. Increased the phosphorylation of FcgRIIa, FAK, Akt, and GSK3β | [73] |
| | | Modulated CRP-XL-induced inside-out signaling to integrin αIIbβ3 and phosphorylation of selective signaling molecules such as AKT, FAK and Src | [74] |

NOX; Nitrous Oxide, ROS; Reactive Oxygen Species, MDA; Malondialdehyde, NO; Nitric Oxide, eNOS; Endothelial NOS, iNOS; Inducible NOS, LPL; Lipoprotein Lipase, HMG-CoA; 3-hydroxy-3-methyl-glutaryl-CoA, LDL; Low-Density Lipoprotein, PPAR-γ; Peroxisome Proliferator-Activated Receptor Gamma, NF-kB; Nuclear Factor Kappa light chain enhancer of activated B cells, Cox-2; Cyclooxygenase-2, PGE2; Prostaglandin E2, TNF-α; Tumor Necrosis Facto-alpha, IL-1β; Interleukin 1 beta, IKK-β; Inhibitor of nuclear factor kappa-B kinase subunit beta, PDGF; Platelet-Derived Growth Factor; NADPH; Nicotinamide Adenine Dinucleotide Phosphate, Syk; Spleen tyrosine kinase, PKC; Protein Kinase C, PLC; Programmable Logic Controller, Akt; Protein kinase B, ERK; Extracellular signal-Regulated Kinases, FcgRIIa; Human Fc gamma RIIA, FAK; Focal Adhesion Kinase, GSK; Glycogen-Synthase Kinase, PVSMCs; Pericytes/Presumptive Vascular Smooth Muscle Cells.

liver cells, which, in turn, this enzyme can convert the oxidizing chrysin and other flavones to toxic compounds [6]. It also illustrated that chrysin significantly shutting down the *de novo* DNA synthesis and inducing cell death [77].

## CONCLUSION

Findings from several experimental studies have indicated that chrysin may be effective against CVDs (Table **1**). Chrysin has been indicated to prevent oxidative stress, inflammation, atherosclerosis, thrombosis, dyslipidemia, and vascular reactivity, proposing a protective effect of chrysin on cardiovascular function. Although the protective effects of chrysin could be due, mostly to its antioxidant activity, it was indicated that chrysin can affect immune and vascular cells to justify their functions through several transcriptional factors involved in CVDs and inflammation. Chrysin acts as a modulator in several steps of inflammation causing endothelial dysfunction, LDL-c accumulation and foam cell formation, smooth muscle cell migration, and inflammatory cytokine expression.

The phenolic hydroxyl groups of chrysin play the main role in scavenging for free radicals, prevention of lipid peroxidation, chelating metal ions, prevention of intestinal cholesterol absorption, cholesterol, and fatty acid synthesis. Furthermore, the hydroxyl groups of chrysin may modulate the production of ROS, inflammatory cytokines, cardiac cell death, platelet activation, vascular endothelial cells injury, dyslipidemia and VSMC proliferation. The data from current studies demonstrate the potential role of chrysin in cardiovascular health. However, it should be considered that the findings from *in vitro* studies, directed to clarify the underlying mechanisms of the cardioprotective effects of chrysin, were gained with chrysin concentrations. Thus, the physiological effects of the *in vitro* findings should be studied. While this compound is indicated to be effective for CVDs treatment in animal models, its effects on human are not clear and its safety is not fully understood. Although, only few articles showed that chrysin exposed inhibition of DNA synthesis and the cell toxicity at very low concentrations. In conclusion, since it is not yet introduced for use as a medicinal agent, clinical trial studies should be done to confirm the safety and efficacy of Chrysin for the clinical approach in patients with cardiovascular disease.

## CONSENT FOR PUBLICATION

Not applicable.

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## CONFLICT OF INTEREST

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

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## REFERENCES

[1] Wolfman, C.; Viola, H.; Paladini, A.; Dajas, F.; Medina, J.H. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from Passifloracoerulea. *Pharmacol. Biochem. Behavior*, **1994**, *47*(1), 1-4.

[2] Yang, M.; Xiong, J.; Zou, Q.; Wang, D.D.; Huang, C.X. Chrysin attenuates interstitial fibrosis and improves cardiac function in a rat model of acute myocardial infarction. *J. Mol. Histol.*, **2018**, *49*(6), 555-565.

[3] Williams, C.A.; Harborne, J.B.; Newman, M.; Greenham, J.; Eagles, J. Chrysin and other leaf exudate flavonoids in the genus Pelargonium. *Phytochemistry*, **1997**, *46*(8), 1349-1353.

[4] Zheng, X.; Zhao, F.F.; Liu, Y.M.; Yao, X.; Zheng, Z.T.; Luo, X.; Liao, D.F. Synthesis and preliminary biological evaluation of chrysin derivatives as potential anticancer drugs. *Med. Chem.*, **2010**, *6*(1), 6-8.

[5] Habtemariam, S. Flavonoids as inhibitors or enhancers of the cytotoxicity of tumor necrosis factor-α in L-929 tumor cells. *J. Nat. Prod.*, **1997**, *60*, 775-778.

[6] Harris, G.K.; Qian, Y.; Leonard, S.S.; Sbarra, D.C.; Shi, X.L. Luteolin and chrysin differentially inhibit cyclooxygenase-2 expression and scavenge reactive oxygen species but similarly inhibit prostaglandin-E-2 formation in RAW 264.7 cells. *J. Nutr.*, **2006**, *136*, 1517-1521.

[7]     Ko, C.H.; Shen, S.C.; Lin, H.Y.; Hou, W.C.; Lee, W.R.; Yang, L.L.; Chen, Y.C. Flavanones structure-related inhibition on TPA-induced tumor promotion through suppression of extracellular signal-regulated protein kinases: Involvement of prostaglandin E-2 in anti-promotive process. *J. Cell. Physiol.*, **2002**, *193*, 93-102.

[8]     Hou, D.X.; Yanagita, T.; Uto, T.; Masuzaki, S.; Fujii, M. Anthocyanidins inhibit cyclooxygenase-2 expression in LPS-evoked macrophages: Structure-activity relationship and molecular mechanisms involved. *Biochem. Pharmacol.*, **2005**, *70*, 417-425.

[9]     Heim, K.E.; Tagliaferro, A.R.; Bobilya, D.J. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.*, **2002**, *13*, 572-584.

[10]    Medina, J.H.; Paladini, A.C.; Wolfman, C.; De Stein, M.L.; Calvo, D.; Diaz, L.E.; Peña, C. Chrysin (5, 7-di-OH-flavone), a naturally-occurring ligand for benzodiazepine receptors, with anticonvulsant properties. *Biochem. Pharmacol.*, **1990**, *40*(10), 2227-2231.

[11]    Tsuji, P.A.; Walle, T. Cytotoxic effects of the dietary flavones chrysin and apigenin in a normal trout liver cell line. *Chem. Biol. Interact.*, **2008**, *171*(1), 37-44.

[12]    Ge, S.; Gao, S.; Yin, T.; Hu, M. Determination of pharmacokinetics of chrysin and its conjugates in wild-type FVB and BCRP1 knock-out mice using a validated LC-MS/MS method. *J. Agricul. Food Chem.*, **2015**, *63*(11), 2902-2910.

[13]    Melekoglu, R.; Ciftci, O.; Eraslan, S.; Alan, S.; Basak, N. The protective effects of glycyrrhetinic acid and chrysin against ischemia-reperfusion injury in rat ovaries. *Biomed. Res. Int.*, **2018**, *2018*, 5421308.

[14]    Sulaiman, G.M.; Jabir, M.S.; Hameed, A.H. Nanoscale modification of chrysin for improved of therapeutic efficiency and cytotoxicity. *Artif. Cells Nanomed. Biotechnol.*, **2018**, *31*, 1-13.

[15]    Wu, T.C.; Chan, S.T.; Chang, C.N.; Yu, P.S.; Chuang, C.H.; Yeh, S.L. Quercetin and chrysin inhibit nickel-induced invasion and migration by downregulation of TLR4/NF-κB signaling in A549 cells. *Chem. Biol. Interact.*, **2018**, *292*, 101-109.

[16]    Zhao, S.; Liang, M.; Wang, Y.; Hu, J.; Zhong, Y.; Li, J.; Huang, K.; Li. Y. Chrysin suppresses vascular endothelial inflammation *via* inhibiting the NF-κB signaling pathway. *J. Cardiovasc. Pharmacol. Ther.*, **2018**, 1074248418810809. DOI: 10.1177/ 1074248418810809.

[17]    Testai, L.; Martelli, A.; Cristofaro, M.; Breschi, M.C.; Calderone, V. Cardioprotective effects of different flavonoids against myocardial ischaemia/ reperfusion injury in Langendorff-perfused rat hearts. *J. Pharm. Pharmacol.*, **2013**, *65*, 750-756.

[18]    Tian, S.S.; Jiang, F.S.; Zhang, K.; Zhu, X.X.; Jin, B.; Lu, J.J.; Ding, Z.S. Flavonoids from the leaves of *Carya cathayensis* Sarg. inhibit vascular endothelial growth factor-induced angiogenesis. *Fitoterapia*, **2014**, *92*, 34-40.

[19]    Bae, Y.; Lee, S.; Kim, S.H. Chrysin suppresses mast cell-mediated allergic inflammation: Involvement of calcium, caspase-1 and nuclear factor-kB. *Toxicol. Appl. Pharmacol.*, **2011**, *254*, 56-64.

[20]    Samarghandian, S.; Azimi-Nezhad, M.; Borji, A.; Hasanzadeh, M.; Jabbari, F.; Farkhondeh, T.; Samini, M. Inhibitory and cytotoxic activities of chrysin on human breast adenocarcinoma cells by induction of apoptosis. *Pharmacogn. Mag.*, **2016**, *12*(4), S436-S440.

[21]    Yao, Y.; Chen, L.; Xiao, J.; Wang, C.; Jiang, W.; Zhang, R.; Hao, J. Chrysin protects against focal cerebral ischemia/reperfusion injury in mice through attenuation of oxidative stress and inflammation. *IJMS*, **2014**, *15*(11), 20913-20926.

[22]    Mantawy, E.M.; El-Bakly, W.M.; Esmat, A.; Badr, A.M.; El-Demerdash, E. Chrysin alleviates acute doxorubicin cardiotoxicity in rats *via* suppression of oxidative stress, inflammation and apoptosis. *Eur. J. Pharmacol.*, **2014**, *728*, 107-118.

[23]    Mehrpour, O.; Aghabiklooei, A.; Abdollahi, M.; Singh, S. Severe hypoglycemia following acute aluminum phosphide (rice tablet) poisoning; a case report and review of the literature. *Acta. Med. Iran*, **2012**, *50*(8), 568-571.

[24]    Rehman, M.U.; Tahir, M.; Khan, A.Q.; Khan, R.; Lateef, A.; Qamar, W.; Sultana, S. Chrysin suppresses renal carcinogenesis *via* amelioration of hyperproliferation, oxidative stress and inflammation: Plausible role of NF-κB. *Toxicol. Lett.*, **2013**, *216*(2-3), 146-158.

[25]    Sharma, P.; Kumari, A.; Gulati, A.; Krishnamurthy, S.; Hemalatha, S. Chrysin isolated from *Pyrus pashia* fruit ameliorates convulsions in experimental animals. *Nutr. Neurosci.*, **2019**, *22*(8), 569-577.

[26]    Uhl, M.; Ecker, S.; Kassie, F.; Lhoste, E.; Chakraborty, A.; Mohn, G.; Knasmüller, S. Effect of chrysin, a flavonoid compound, on the mutagenic activity of 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine (PhIP) and benzo (a) pyrene (B (a) P) in bacterial and human hepatoma (HepG2) cells. *Arch. Toxicol.*, **2003**, *77*(8), 477-484.

[27]    Mehrpour, O.; Karrari, P.; Abdollahi, M. Chronic lead poisoning in Iran; a silent disease. *Daru*, **2012**, *20*(1), 8.

[28]    Souza, L.C.; Antunes, M.S.; Borges Filho, C.; Del Fabbro, L.; De Gomes, M.G.; Goes, A.T.R.; Jesse, C.R. Flavonoid chrysin prevents age-related cognitive decline *via* attenuation of oxidative stress and modulation of BDNF levels in aged mouse brain. *Pharmacol. Biochem. Behav.*, **2015**, *134*, 22-30.

[29]    Goodarzi, F.; Mehrpour, O.; Eizadi-Mood, N. View correspondence (jump link). A study to evaluate factors associated with seizure in tramadol poisoning in Iran. *Indian J. Forensic Med. Toxicol.*, **2011**, *5*(2), 66-69.

[30]    Karrari, P.; Mehrpour, O.; Afshari, R.; Keyler, D. Pattern of illicit drug use in patients referred to addiction treatment centres in Birjand, Eastern Iran. *J. Pak. Med. Assoc.*, **2013**, *63*(6), 711-716.

[31]    Alinejad, S.; Aaseth, J.; Abdollahi, M.; Hassanian-Moghaddam, H.; Mehrpour, O. Clinical aspects of opium adulterated with lead in Iran: A review. *Basic Clin. Pharmacol. Toxicol.*, **2018**, *122*(1), 56-64.

[32]    Mehrpour, O.; Amouzeshi, A.; Dadpour, B.; Oghabian, Z.; Zamani, N.; Amini, S.; Hoffman, R.S. Successful treatment of cardiogenic shock with an intraaortic balloon pump following aluminium phosphide poisoning. *Arh. Hig. Rada Toksikol.*, **2014**, *65*(1), 121-126.

[33]    Samarghandian, S.; Ohata, H.; Yamauchi, N.; Shibasaki, T. Corticotropin-releasing factor as well as opioid and dopamine are involved in tail-pinch-induced food intake of rats. *Neuroscience*, **2003**, *116*(2), 519-524.

[34]    Siti, H.N.; Kamisah, Y.; Kamsiah, J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *Vasc. Pharmacol.*, **2015**, *71*, 40-56.

[35]    Samarghandian, S.; Azimi-Nezhad, M.; Farkhondeh, T. Catechin treatment ameliorates diabetes and its complications in streptozotocin-induced diabetic rats. *Dose Response*, **2017**, *15*(1), 1559325817691158.

[36]    Patel, R.V.; Mistry, B.; Syed, R.; Rathi, A.K.; Lee, Y.J.; Sung, J.S.; Shinf, H.S.; Keum, Y.S. Chrysin-piperazine conjugates as antioxidant and anticancer agents. *Eur. J. Pharm. Sci.*, **2016**, *88*, 166-77.

[37]    Hajzadeh, M.A.R.; Rajaei, Z.; Shafiee, S.; Alavinejhad, A.; Samarghandian, S.; Ahmadi, M. Effect of barberry fruit (*Berberis Vulgaris*) on serum glucose and lipids in streptozotocin-diabetic rats. *Pharmacol. Online*, **2011**, *1*, 809-817.

[38]    Berliner, J.A.; Navab, M.; Fogelman, A.M.; Frank, J.S.; Demer, L.L.; Edwards, P.A.; Lusis, A.J. Atherosclerosis: Basic mechanisms: Oxidation, inflammation, and genetics. *Circulation*, **1995**, *91*(9), 2488-2496.

[39]    Missassi, G.; Dos Santos Borges, C.; De Lima Rosa, J.; Villela e Silva, P.; Da Cunha Martins, A.; Barbosa, F.; De Grava Kempinas, W. Chrysin administration protects against oxidative damage in varicocele-induced adult rats. *Oxid. Med. Cell Longev.*, **2017**, *2017*.

[40]    Sharma, P.; Kumari, A.; Gulati, A.; Krishnamurthy, S.; Hemalatha, S. Chrysin isolated from *Pyruspashia* fruit ameliorates convulsions in experimental animals. *Nutr. Neurosci.*, **2019**, *22*(8), 569-577.

[41]    Anandhi, R.; Annadurai, T.; Anitha, T.S.; Muralidharan, A.R.; Najmunnisha, K.; Nachiappan, V.; Thomas, P.A.; Geraldine, P. Antihypercholesterolemic and antioxidative effects of an extract of the oyster mushroom, Pleurotusostreatus, and its major constituent, chrysin, in Triton WR-1339-induced hypercholesterolemic rats. *J. Physiol. Biochem.*, **2013**, *69*(2), 313-323.

[42]    Yang, J.R. Effect of chrysin on expression of NOX4 and NF-κB in right ventricle of monocrotaline-induced pulmonary arterial hypertension of rats. *Yao XueXueBao*, **2015**, *50*(9), 1128-1134.

[43]    Li, X.W.; Wang, X.M.; Li, S.; Yang, J.R. Effects of chrysin (5, 7-dihydroxyflavone) on vascular remodeling in hypoxia-induced pulmonary hypertension in rats. *Chin. Med.*, **2015**, *10*(1), 4.

[44]    Veerappan, R.; Senthilkumar, R. Chrysin enhances antioxidants and oxidative stress in L-NAME-induced hypertensive rats. *Int. J. Nutr. Pharmacol. Neurol. Dis.*, **2015**, *5*(1), 20.

[45]    Hecker, M.; Preiß, C.; Klemm, P.; Busse, R. Inhibition by antioxidants of nitric oxide synthase expression in murine macrophages:

Role of nuclear factor kB and interferon regulatory factor 1. *Brit. J. Pharmacol.*, **1996**, *118*(8), 2178-2184.

[46]    Anandhi, R.; Thomas, P.A.; Geraldine, P. Evaluation of the anti-atherogenic potential of chrysin in Wistar rats. *Mol. Cell Biochem.*, **2014**, *385*(1-2), 103-113.

[47]    El-Bassossy, H.M.; Abo-Warda, S.M.; Fahmy, A. Chrysin and luteolin attenuate diabetes‑induced impairment in endothelial‑dependent relaxation: Effect on lipid profile, AGEs and NO generation. *Phytother. Res.*, **2013**, *27*(11), 1678-1684.

[48]    Samarghandian, S.; Azimi-Nezhad, M.; Samini, F.; Farkhondeh, T. Chrysin treatment improves diabetes and its complications in liver, brain, and pancreas in streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol.*, **2016**, *94*(4), 388-393.

[49]    Ramírez-Espinosa, J.J.; Saldaña-Ríos, J.; García-Jiménez, S.; Villalobos-Molina, R.; Ávila-Villarreal, G.; Rodríguez-Ocampo, A.N.; Estrada-Soto, S. Chrysin induces antidiabetic, antidyslipidemic and anti-inflammatory effects in athymic nude diabetic mice. *Molecules*, **2017**, *23*(1), 67.

[50]    Paulo, L.L.; Cruz, J.C.; Zhuge, Z.; Carvalho-Galvão, A.; Brandão, M.C.; Diniz, T.F.; Montenegro, M.F. The novel organic mononitrate NDHP attenuates hypertension and endothelial dysfunction in hypertensive rats. *Redox Biol.*, **2018**, *15*, 182-191.

[51]    Veerappan, R.; Malarvili, T. Chrysin pretreatment improves angiotensin system, cGMP concentration in L-NAME induced hypertensive rats. *Ind. J. Clin. Biochem.*, **2019**, *34*(3), 288-295.

[52]    Duarte, J.; Jiménez, R.; Villar, I.C.; Pérez-Vizcaíno, F.; Jiménez, J.; Tamargo, J. Vasorelaxant effects of the bioflavonoid chrysin in isolated rat aorta. *Plantamedica*, **2001**, *67*(6), 567-569.

[53]    Villar, I.C.; Galisteo, M.; Vera, R.; O'Valle, F.; García-Saura, M. F.; Zarzuelo, A.; Duarte, J. Effects of the dietary flavonoid chrysin in isolated rat mesenteric vascular bed. *J. Vasc. Res.*, **2004**, *41*(6), 509-516.

[54]    Li, X.W.; Wang, X.M.; Li, S.; Yang, J.R. Effects of chrysin (5, 7-dihydroxyflavone) on vascular remodeling in hypoxia-induced pulmonary hypertension in rats. *Chin. Med.*, **2015**, *10*(1), 4.

[55]    Ajay, M.; Gilani, A.U.H.; Mustafa, M.R. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. *Life Sci.*, **2003**, *74*(5), 603-612.

[56]    Calderone, V.; Chericoni, S.;Martinelli, C.;Testai, L.; Nardi, A.; Morelli, I.; Martinotti, E. Vasorelaxing effects of flavonoids: Investigation on the possible involvement of potassium channels. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **2004**, *370*(4), 290-298.

[57]    Golia, E.; Limongelli, G.; Natale, F.; Fimiani, F.; Maddaloni, V.; Pariggiano, I.; Di Palma, G. Inflammation and cardiovascular disease: From pathogenesis to therapeutic target. *Curr. Atheroscler. Rep.*, **2014**, *16*(9), 435.

[58]    Singh, J.; Chaudhari, B.P.; Kakkar, P. Baicalin and chrysin mixture imparts cytoprotection against methylglyoxal induced cytotoxicity and diabetic tubular injury by modulating RAGE, oxidative stress and inflammation. *Environ. Toxicol. Pharmacol.*, **2017**, *50*, 67-75.

[59]    Harris, G.K.; Qian, Y.; Leonard, S.S.; Sbarra, D.C.; Shi, X. Luteolin and chrysin differentially inhibit cyclooxygenase-2 expression and scavenge reactive oxygen species but similarly inhibit prostaglandin-E2 formation in RAW 264.7 cells. *J. Nutr.*, **2006**, *136*(6), 1517-1521.

[60]    Lee, S.H.; Kim, Y.J.; Kwon, S.H.; Lee, Y.H.; Choi, S.Y.; Park, J.S.; Kwon, H.J. Inhibitory effects of flavonoids on TNF-α-induced IL-8 gene expression in HEK 293 cells. *BMB reports*, **2009**, *42*(5), 265-270.

[61]    Li, X.W.; Guo, B.; Shen, Y.Y.; Yang, J.R. Effect of chrysin on expression of NOX4 and NF-κB in right ventricle of monocrotaline-induced pulmonary arterial hypertension of rats. *Yao xuexuebao= Actapharmaceutica Sinica*, **2015**, *50*(9), 1128-1134.

[62]    Panahi, G.; Pasalar, P.; Zare, M.; Rizzuto, R.; Meshkani, R. High glucose induces inflammatory responses in HepG2 cells *via* the oxidative stress-mediated activation of NF-κB, and MAPK pathways in HepG2 cells. *Arch. Physiol. Biochem.*, **2018**, 1-7.

[63]    Saum, K.; Campos, B.; Celdran-Bonafonte, D.; Nayak, L.; Sangwung, P.; Thakar, C.; Owens, A. P. Uremic advanced glycation end products and protein-bound solutes induce endothelial dysfunction through suppression of Krüppel-like factor 2. *J. Am. Heart Assoc.*, **2018**, *7*(1), e007566.

[64]    Rani, N.; Bharti, S.; Bhatia, J.; Nag, T.C.; Ray, R.; Arya, D.S. Chrysin, a PPAR-γ agonist improves myocardial injury in diabetic rats through inhibiting AGE-RAGE mediated oxidative stress and inflammation. *Chem. Biol. Interact.*, **2016**, *250*, 59-67.

[65]    Yang, L.; Gao, L.; Nickel, T.; Yang, J.; Zhou, J.; Gilbertsen, A.; Gourley, G. R. Lactate promotes synthetic phenotype in vascular smooth muscle cells novelty and significance. *Circ. Res.*, **2017**, *121*(11), 1251-1262.

[66]    Kappert, K.1.; Sparwel, J.; Sandin, A.; Seiler, A.; Siebolts, U.; Leppänen, O.; Rosenkranz, S.; Ostman, A. Antioxidants relieve phosphatase inhibition and reduce PDGF signaling in cultured VSMCs and in restenosis. *Arteriosc. Throm. Vasc. Biol.*, **2006**, *26*(12), 2644-2651.

[67]    Lo, H.M.; Wu, M.W.; Pan, S.L.; Peng, C.Y.; Wu, P.H.; Wu, W.B. Chrysin restores PDGF-induced inhibition on protein tyrosine phosphatase and reduces PDGF signaling in cultured VSMCs. *J. Nutr. Biochem.*, **2012**, *23*(6), 667-678.

[68]    Roe, A.; Frisk, M.; E Louch, W. Targeting cardiomyocyte Ca2 homeostasis in heart failure. *Curr. Pharm. Des.*, **2015**, *21*(4), 431-448.

[69]    Anghel, N.; Cotoraci, C.; Ivan, A.; Suciu, M.; Herman, H.; Balta, C.; Hermenean, A. Chrysin attenuates cardiomyocyte apoptosis and loss of intermediate filaments in a mouse model of mitoxantrone cardiotoxicity. *Histol. Histopathol.*, **2015**, *30*(12), 1465-1475.

[70]    Davì, G.; Patrono, C. Platelet activation and atherothrombosis. *New England J. Med.*, **2007**, *357*(24), 2482-2494.

[71]    Gibbins, J.M. Platelet adhesion signalling and the regulation of thrombus formation. *J. Cell Sci.*, **2004**, *11*(16), 3415-3425.

[72]    Vaiyapuri, S.; Ali, M.S.; Moraes, L.A.; Sage, T.; Lewis, K.R.; Jones, C.I.; Gibbins, J.M. Tangeretin regulates platelet function through inhibition of phosphoinositide 3-kinase and cyclic nucleotide signaling significance. *Arteriosc. Throm. Vasc. Biol.*, **2013**, *33*(12), 2740-2749.

[73]    Liu, G.; Xie, W.; He, A.D.; Da, X.W.; Liang, M.L.; Yao, G.Q.; Ming, Z.Y. Antiplatelet activity of chrysin *via* inhibiting platelet αIIbβ3-mediated signaling pathway. *Mol. Nutr. Food Res.*, **2016**, *60*(9), 1984-1993.

[74]    Ravishankar, D.; Salamah, M.; Attina, A.; Pothi, R.; Vallance, T. M.; Javed, M.; Shankland, K. Ruthenium-conjugated chrysin analogues modulate platelet activity, thrombus formation and haemostasis with enhanced efficacy. *Sci. Rep.*, **2017**, *7*(1), 5738.

[75]    Walle, T.; Otake, Y.; Brubaker, J.; Walle, U.; Halushka P. Disposition and metabolism of the flavonoid chrysin in normal volunteers. *Br. J. Clin. Pharmacol.*, **2001**, *51*, 143-146.

[76]    Saarinen, N.; Joshi, S.C.; Ahotupa, M.; Li, X.; Ämmälä, J.; Mäkelä, S.; Santti, R. No evidence for the *in vivo* activity of aromatase-inhibiting flavonoids. *J. Steroid Biochem. Mol. Biol.*, **2001**, *78*, 213-239.

[77]    Gambelunghe, C.; Rossi, R.; Sommavilla, M.; Ferranti, C.; Rossi, R.; Ciculi, C.; Gizzi, S.; Micheletti, A.; Rufini, S. Effects of chrysin on urinary testosterone levels in human males. *J. Med. Food*, **2003**, *6*, 387-390.