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Cytoprotective effects of euxanthone against ox-LDL-induced endothelial cell injury is mediated via Nrf2

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ABSTRACT

Aim: Atherosclerosis (AS) is a chronic condition of the arterial vessels and a risk factor for myocardial infarction and stroke. Euxanthone is a xanthone compound extracted from *Polygala caudata*, and shows vasodilatory action. The aim of this study was to determine the potential pharmacological effects of euxanthone against oxidized low-density lipoprotein (ox-LDL)-induced endothelial cell injury.

Material and methods: Human umbilical vein endothelial cells (HUVECs) were exposed to ox-LDL, following pre-treatment with different concentrations of euxanthone. Viability, apoptosis and DNA fragmentation were respectively assessed by CCK-8 assay, Annexin-V/PI staining and TdT-mediated dUTP Nick-End Labeling (TUNEL) assay. The cellular levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were analyzed by enzyme linked immune-sorbent assays (ELISA), and reactive oxygen species (ROS) levels using dichlorodihydrofluorescin diacetate (DCFH) staining. Quantitative RT-PCR and Western blotting were respectively used to analyze the expression levels of specific mRNAs and proteins. HUVECs were transfected with Nrf2 siRNA to induce knockdown of the latter.

Key findings: Euxanthone pre-treatment rescued the HUVECs from ox-LDL-induced cytotoxicity, apoptosis and DNA fragmentation in a dose-dependent manner. In addition, euxanthone also significantly reversed ox-LDL-triggered loss of mitochondrial membrane potential (MMP), cytochrome C release from mitochondria to cytosol, cleavage of caspase-3 and PARP, and increase in Bax/Bcl-2 ratio. Pre-treatment with euxanthone markedly suppressed ox-LDL-induced ROS generation and inhibition of antioxidant enzymes, as well as the up-regulation of pro-inflammatory factors like MCP-1, IL-1β and TNF-α in the HUVECs. Euxanthone up-regulated and activated Nrf2 by repressing Keap1, and increased the expression of its downstream genes HO-1 and NQO-1. Nrf2 knockdown abrogated the cyto-protective, anti-apoptotic, anti-oxidant and anti-inflammatory effects of euxanthone in ox-LDL-treated HUVECs. Finally, euxanthone activated Nrf2 via the MAPK pathway and blocking the latter likewise negated the protective effects of euxanthone against cell ox-LDL.

Significance: Euxanthone protected HUVECs against the oxidative and inflammatory damage induced by ox-LDL, indicating its potential as a novel therapeutic agent for AS.

1. Introduction

Atherosclerosis (AS) refers to the chronic inflammation of the arterial vessel walls, and plays a key role in the pathophysiology of cardiovascular diseases such as myocardial infarction and stroke [1,2]. Multiple factors contribute to atherogenesis, including excessive oxidative stress, lipoprotein modifications, immune reactivity and inflammation, which ultimately lead to endothelial dysfunction, an early event in atherogenesis [3]. A major trigger of endothelial dysfunction is oxidized low-density lipoprotein (ox-LDL)-induced cell injury [3], which decreases nitric oxide synthesis, enhances secretion of adhesion molecules and facilitates the production of chemo-attractants and growth factors, which altogether facilitate atherogenesis [4].

Nuclear factor erythroid 2-related factor 2 (Nrf2), a widely expressed transcription factor, is the master regulator of redox stress response [5]. In addition, a number of studies have shown the

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Table 1
Primer sequences of qRT-PCR.

Gene	Sequence	
MCP-1	Forward primer	5′-CCCCAGTCACCTGCTGTTAT-3′
	Reverse primer	5′-TGGAATCCTGAACCCACTTC-3′
IL-1β	Forward primer	5′-CCACAGACCTTCCAGGAGAAT-3′
	Reverse primer	5′-GTGCACATAAGCCTCGTTATCC-3′
TNF-α	Forward primer	5′-ACCTCTCTCTAATCAGCCCTCT-3′
	Reverse primer	5′-GGGTTTGCTACAACATGGGCTA-3′
Nrf2	Forward primer	5′-CAGTCAGCGACGGAAAGAGTA-3′
	Reverse primer	5′-TGTGGGCAACCTGGGAGTAG-3′
HO-1	Forward primer	5′-GGCAGAGGGTGATAGAAGAGG-3′
	Reverse primer	5′-AGCTCCTGCAACTCCTCAAA-3′
NQO-1	Forward primer	5′-CAGCTCACCGAGAGCCTAGT-3′
	Reverse primer	5′-GAGTGAGCCAGTACGATCAGTG-3′
GAPDH	Forward primer	5′-GCACCGTCAAGGCTGAGAAC-3′
	Reverse primer	5′-GGATGCAGGGATGATGTTCT-3′

involvement of Nrf2 in the pathogenesis of AS [6]. Nrf2 activation protects against endothelium damage, and several natural compounds inhibit oxidative stress in the endothelial cells via Nrf2 [7,8]. Therefore, Nrf2 is a promising therapeutic target in the atherosclerotic endothelium.

Several plant-derived compounds have shown promising cardioprotective effects, and resulted in a decreased incidence rate of cardiovascular disease [9]. Euxanthone is a xanthone compound extracted from *Polygala caudata*, a medicinal herb widely distributed in the southwest part of China and used for treating cough and anxiety [10,11]. Euxanthone also showed a vasodilatory effect on the rat aorta [12,13], although its potential beneficial effect on AS remains unclear. We used the HUVECs as the in vitro model to explore the effects of euxanthone on endothelial cell injury following ox-LDL exposure, and elucidate the underlying molecular mechanisms.

2. Materials and methods

2.1. Cell culture

HUVECs were obtained from the Cell bank of Chinese Academy of Sciences (Shanghai, China). The cells were maintained in F-12 K medium (ATCC, VA, USA) supplemented with 10% fetal bovine serum (FBS), 0.1 mg/ml heparin, and 1% antibiotic-antimycotic solution at 37 °C in a CO_2 humidified incubator. To establish endothelial cells injury model, HUVECs were pretreated with different concentrations of ox-LDL (0, 20, 50, and 100 mg/l) (UBC-ox-LDL5, Union Biology Co. Ltd, Beijing, China) for 24 h.

2.2. Cell viability assay

CCK-8 assay was performed as previously described [14]. Briefly, HUVEC cells were seeded into 96-well plates at the density of 5×10^3 cells/ml. Following treatment, fresh medium containing 10% (v/v) CCK-8 reaction solution was added, and the cells were incubated for another 2 h. The absorbance of the wells was measured at 450 nm using a microplate reader.

2.3. Annexin V/PI staining

Apoptosis was detected using an Annexin V-FITC/PI apoptosis kit (BD pharmingen, NJ, USA) according to the manufacturer's instructions. The stained samples were acquired using a flow cytometer (Beckman Coulter lnc, FL, USA), and the percentage of apoptotic cells was analyzed.

2.4. TUNEL assay

Suitably treated HUVECs were stained with the TUNEL labeling kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. At least 300 cells were counted per field in five randomly selected fields under 100× magnification for every sample, and the percentage of the TUNEL positive cells was calculated.

2.5. Mitochondrial depolarization assay

Following incubation with Soy B for 48 h, the change in MMP was evaluated by JC-1 staining as previously described [15].

2.6. Detection of MDA and antioxidant enzyme levels

Total protein was extracted from the HUVECs using the RIPA lysis buffer (Beyotime, Shanghai, China), and the levels of SOD, MDA, CAT and GST-PX were detected using total SOD assay kit, lipid peroxidation MDA assay kit, CAT assay kit and total GSH-PX assay kit respectively (Beyotime), according to the manufacture's protocols.

2.7. Measurement of ROS levels

An OxiSelect In Vitro ROS/RNS Assay Kit (Cell Biolabs, San Diego, CA, USA) was used to determine the ROS levels in each sample [16]. Briefly, cells were lysed with a lysis buffer, and the total protein content in the lysates were measured using the BCA assay (Thermo Scientific, Shanghai, China). The catalyst solution and dichlorodihydrofluorescin (DCFH) solution were then added to each sample in a 96-well plate. After incubating for 20 min in the dark, the fluorescence signal was detected with a fluorometric microscopy at the excitation of 480 nm and emission 530 nm.

2.8. Western blotting

A commercially available lysis buffer (Beyotime) was used to extract nuclear and cytoplasmic proteins as previously described [17]. Equal amount of proteins per sample were separated by sodium dodecyl sulfate-polyacrylamide electrophoresis, and transferred to polyvinylidene fluoride (PVDF) membranes. The blots were incubated overnight with the primary antibodies (including GAPDH as the internal control) at 4 °C, followed by goat anti-rabbit IgG-HRP (Beyotime, Shanghai, China) secondary antibody at room temperature for 1 h. The positive bands were visualized using enhanced BeyoECL Star (#P0018AM, Beyotime Biotechnology, Shanghai, China) according to the manufacturer's protocols.

2.9. Quantitative real-time PCR (qRT-PCR) assay

Total RNA was extracted using the Trizol reagent (Invitrogen) and then reversely transcribed to cDNA using PrimeScript Master Mix (TaKaRa, Dalian, China). The levels of Nrf2, HO-1, NQO-1 and GAPDH (internal control) mRNAs were analyzed using the SYBR RT-PCR Kit (Takara). The primers are listed in Table 1 [18,19]. The relative expression of the different genes was quantified using the $^{\Delta\Delta}$Ct method.

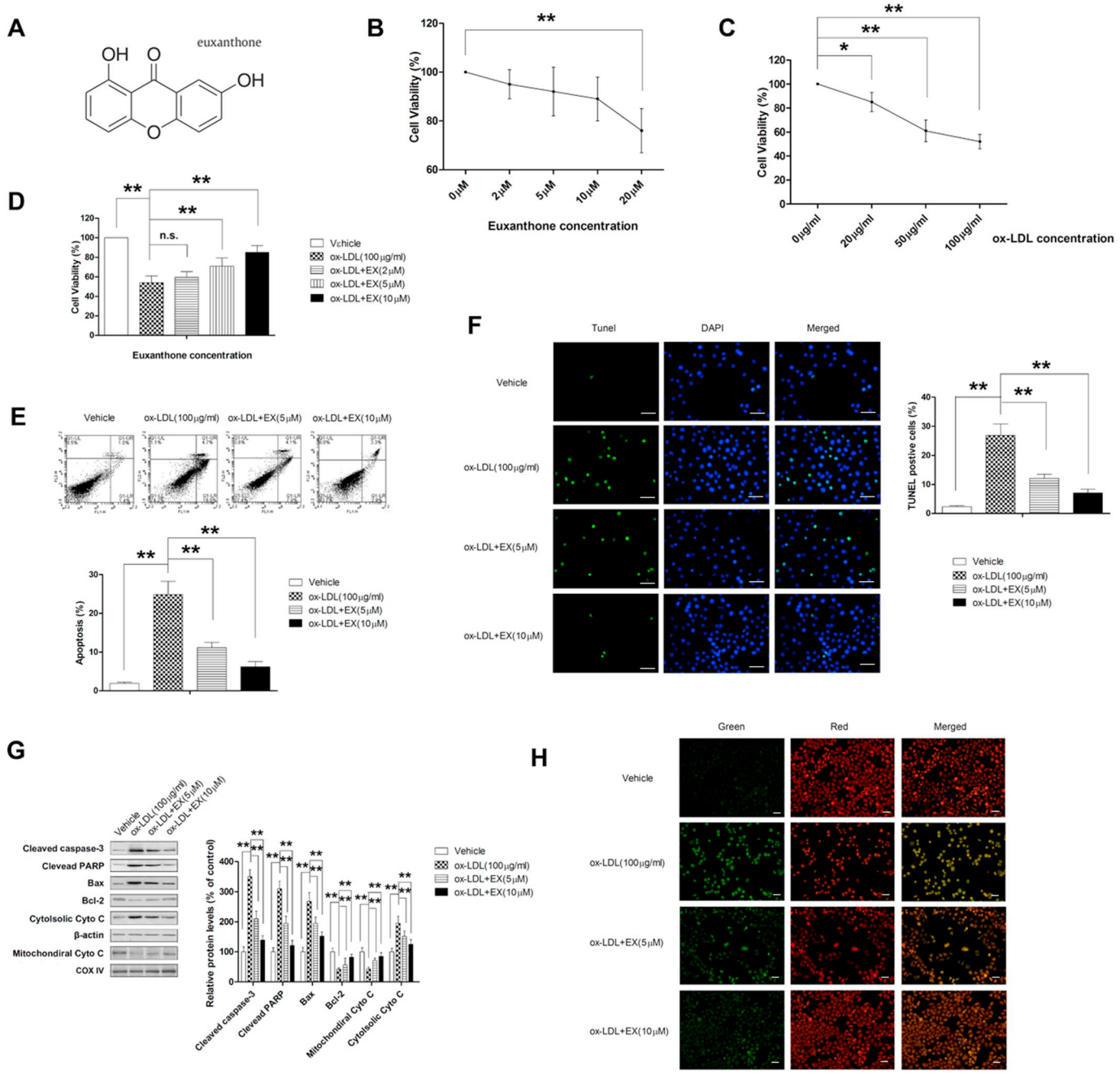

Fig. 1. Euxanthone alleviated ox-LDL-induced cytotoxicity and apoptosis in HUVECs. (A) Chemical structure of euxanthone. (B) Viability of HUVECs treated with 20 μM euxanthone for 24 h. (C) Ox-LDL decreased viability of HUVECs in a dose-dependent manner. Pre-treatment with 5 and 10 μM euxanthone for 6 h rescued HUVECs from ox-LDL-induced cytotoxicity (D), apoptosis (E) and DNA fragmentation (F). (G) Euxanthone suppressed cleavage of caspase-3 and PARP, up-regulated Bcl-2, down-regulated Bax, and inhibited cytochrome C release from mitochondria to cytosol in ox-LDL-treated HUVECs. (H) Euxanthone restored loss of MMP in ox-LDL-treated HUVECs. Scale bar = 200 μm. *P < 0.05, **P < 0.01.

2.10. Knockdown of Nrf2

HUVECs were cultured in 6-well plates, and transfected with Nrf2 siRNA (OriGene Technologies, Beijing, China) or the scrambled siRNA using Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's instructions. Forty-eight hours after transfection, the Nrf2 mRNA and protein levels were detected using qRT-PCR and Western blotting.

2.11. Statistical analysis

Data are presented as means ± SD. Multiple groups were compared

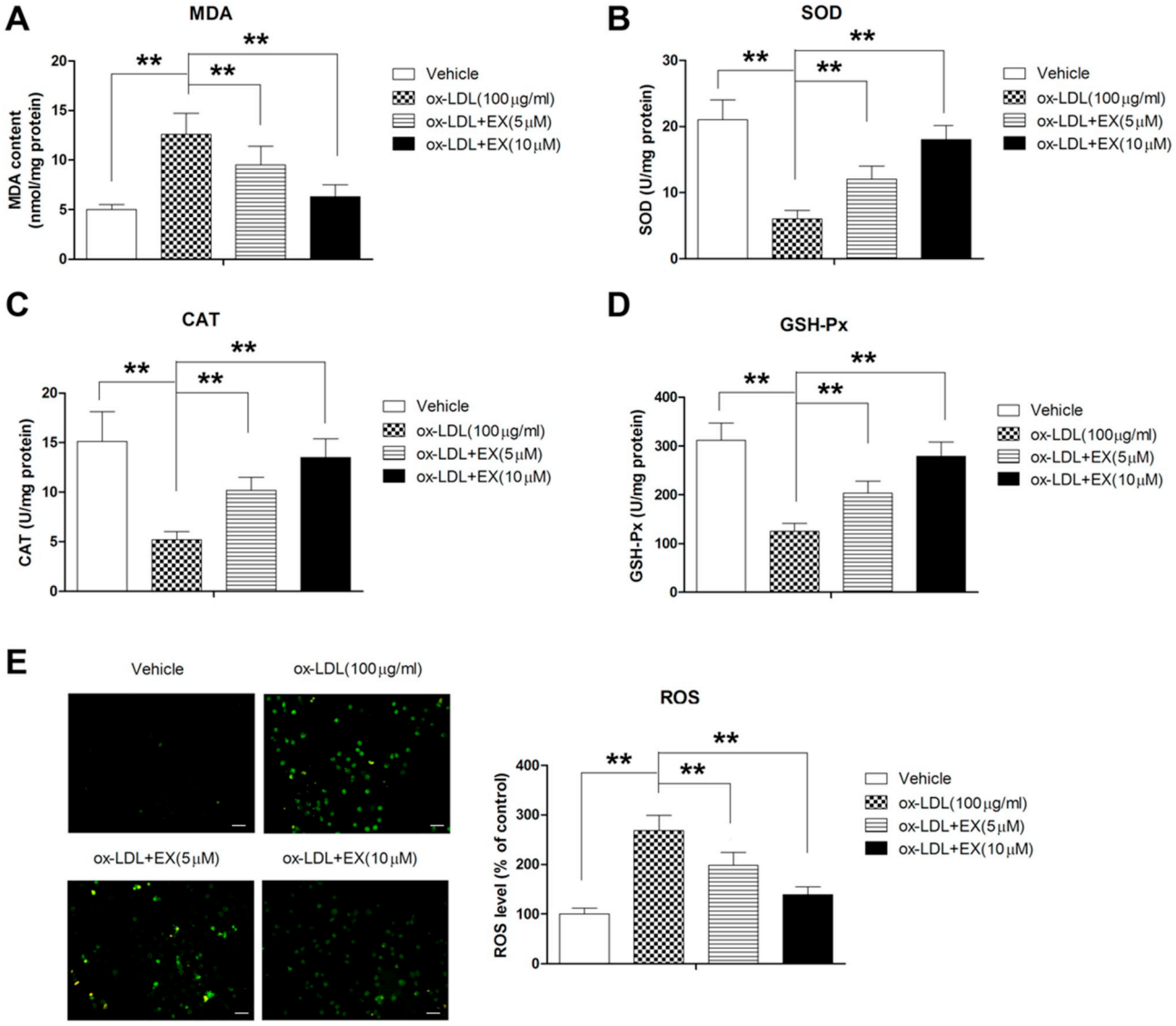

Fig. 2. Euxanthone protects HUVECs against ox-LDL-induced oxidative damage. Euxanthone pre-treatment decreased MDA levels (A), elevated the levels of SOD (B), CAT (C) and GSH-Px (D), and suppressed ROS levels (E) in HUVECs treated with ox-LDL. Scale bar = 200 μm. **P < 0.01.

by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA). P values < 0.05 were considered statistically significant.

3. Results

3.1. *Euxanthone antagonizes ox-LDL-promoted cell death and apoptosis in HUVECs*

The chemical structure of euxanthone is shown in Fig. 1A. Ox-LDL treatment for 24 h significantly reduced the viability of HUVECs in a dose-dependent manner (Fig. 1C). Since 20 μM euxanthone was toxic to the HUVECs (< 80% viability after 24 h, P < 0.01 vs. control; Fig. 1B), we pre-treated the cells with 2 μM, 5 μM or 10 μM euxanthone for 6 h before ox-LDL exposure. While 2 μM euxanthone had no effect on the viability of HUVECs, the higher doses were able to ameliorate the cytotoxic effects of ox-LDL (Fig. 1D). Furthermore, 5 μM and 10 μM euxanthone reduced the percentage of apoptotic HUVECs to 10% and 5% respectively following ox-LDL exposure, compared to the 20% apoptosis rate in the untreated cells (Fig. 1E). ox-LDL also increased the content of fragmented DNA, as shown by TUNEL staining, which was markedly decreased in the HUVECs pre-treated with euxanthone (Fig. 1F). As shown in Fig. 1G, euxanthone also blocked ox-LDL-mediated caspase-3 and PARP cleavage in the HUVECs, thereby reversing mitochondrial dysfunction that plays a key role in ox-LDL-induced apoptosis [20]. We observed loss of MMP, cytochrome C release from mitochondria to cytosol, and increased Bax/Bcl-2 ratio in the HUVECs following ox-LDL treatment (Fig. 1G and H). However, pre-treatment with euxanthone markedly attenuated the mitochondrial dysfunction.

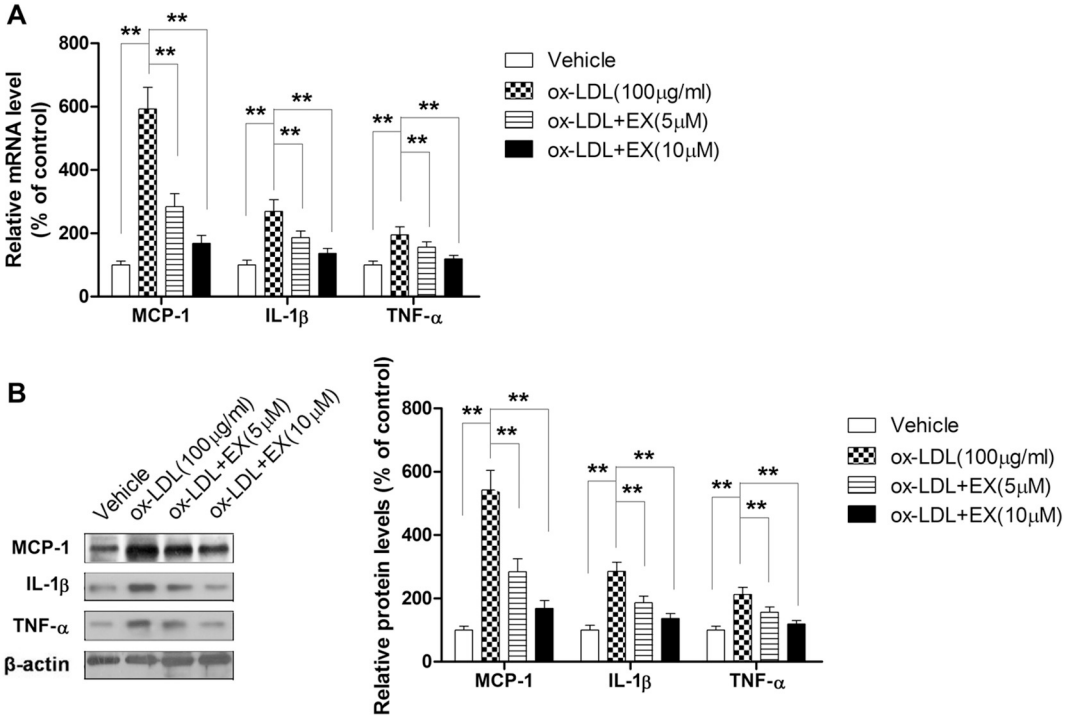

Fig. 3. Euxanthone inhibits the inflammatory response activated by ox-LDL in HUVECs. Euxanthone down-regulated the levels of MCP-1, IL-1β and TNF-α mRNA (A) and proteins (B) in HUVECs. **P < 0.01.

3.2. Euxanthone weakens ox-LDL-induced oxidative stress in HUVECs

As shown in Fig. 2A, ox-LDL increased MDA levels in the HUVECs by > 2 folds compared to the control cells, which was significantly reduced by 5 and 10 μM euxanthone. In addition, euxanthone pre-treatment also markedly restored the SOD levels in HUVECs (Fig. 2B), which was decreased to < 40% by ox-LDL, as well as that of CAT and GSH-Px (Fig. 2C and D). Finally, euxanthone pre-treatment decreased the intracellular ROS levels that were markedly increased by ox-LDL (Fig. 2E). Taken together, euxanthone protected HUVECs against ox-LDL-induced oxidative stress by restoring the antioxidant system and decreasing ROS levels.

3.3. Euxanthone inhibits inflammation in HUVECs treated with ox-LDL

Ox-LDL markedly increased the expression levels of the pro-inflammatory MCP-1, IL-1β and TNF-α in HUVECs, which was reduced by euxanthone in a dose-dependent manner (Fig. 3A and B). Therefore, euxanthone showed a potent anti-inflammatory effect in HUVECs exposed to ox-LDL.

3.4. Euxanthone upregulates Nrf2 and promotes its nuclear translocation

As shown in Fig. 4A, treatment with euxanthone resulted in a dose-dependent increase in Nrf2 mRNA levels in the HUVECs. In addition, euxanthone significantly increased the nuclear content of Nrf2 protein, without affecting its cytoplasmic levels (Fig. 4B). Keap1, a repressor of Nrf2, was significantly reduced by euxanthone (Fig. 4B), indicating that euxanthone regulated Nrf2 by down-regulating Keap1. HO-1 and NQO-1 are key downstream genes of Nrf2, and mediate its cardio-protective effects [21]. We found that euxanthone treatment up-regulated both HO-1 and NQO-1 in a dose-dependent manner (Fig. 4C and D), further confirming that euxanthone activated Nrf2 in the HUVECs. Furthermore, euxanthone up-regulated Nrf2 and promoted its nuclear translocation (P < 0.01 vs. ox-LDL; Fig. 4E), in addition to down-regulating Keap1 (Fig. 4F) and up-regulating HO-1 and NQO-1 (Fig. 4G and H) in the ox-LDL-treated HUVECs.

3.5. Euxanthone protects against ox-LDL-induced injury via Nrf2

To determine the role of Nrf2 in the cyto-protective effects of euxanthone, HUVECs were transfected with Nrf2-targeting siRNA, which repressed Nrf2 levels to ~30% (Fig. 5A). Nrf2 knockdown abolished the protective effects of euxanthone against ox-LDL-induced decrease in viability (Fig. 5B) and apoptosis (Fig. 5C). In addition, euxanthone failed to modulate the levels of apoptotic proteins (Fig. 5D), as well as the redox factors (Fig. 6A–D) and ROS levels (Fig. 6E) in Nrf2-knockdown HUVECs. As shown in Fig. 7A and B, Nrf2 knockdown also abolished the repressive effects of euxanthone on MCP-1, IL-1β and TNF-α. Taken together, Nrf2 mediates the cyto-protective effects of euxanthone.

3.6. Euxanthone activates Nrf2 signaling via the MAPK signaling pathway

The MAPK pathway has been previously implicated in the activation of Nrf2 [22]. As shown in Fig. 8A, ox-LDL significantly activated ERK, p38 and JNK, while pre-treatment with euxanthone only elevated the levels of phosphorylated ERK. The involvement of ERK in the regulatory

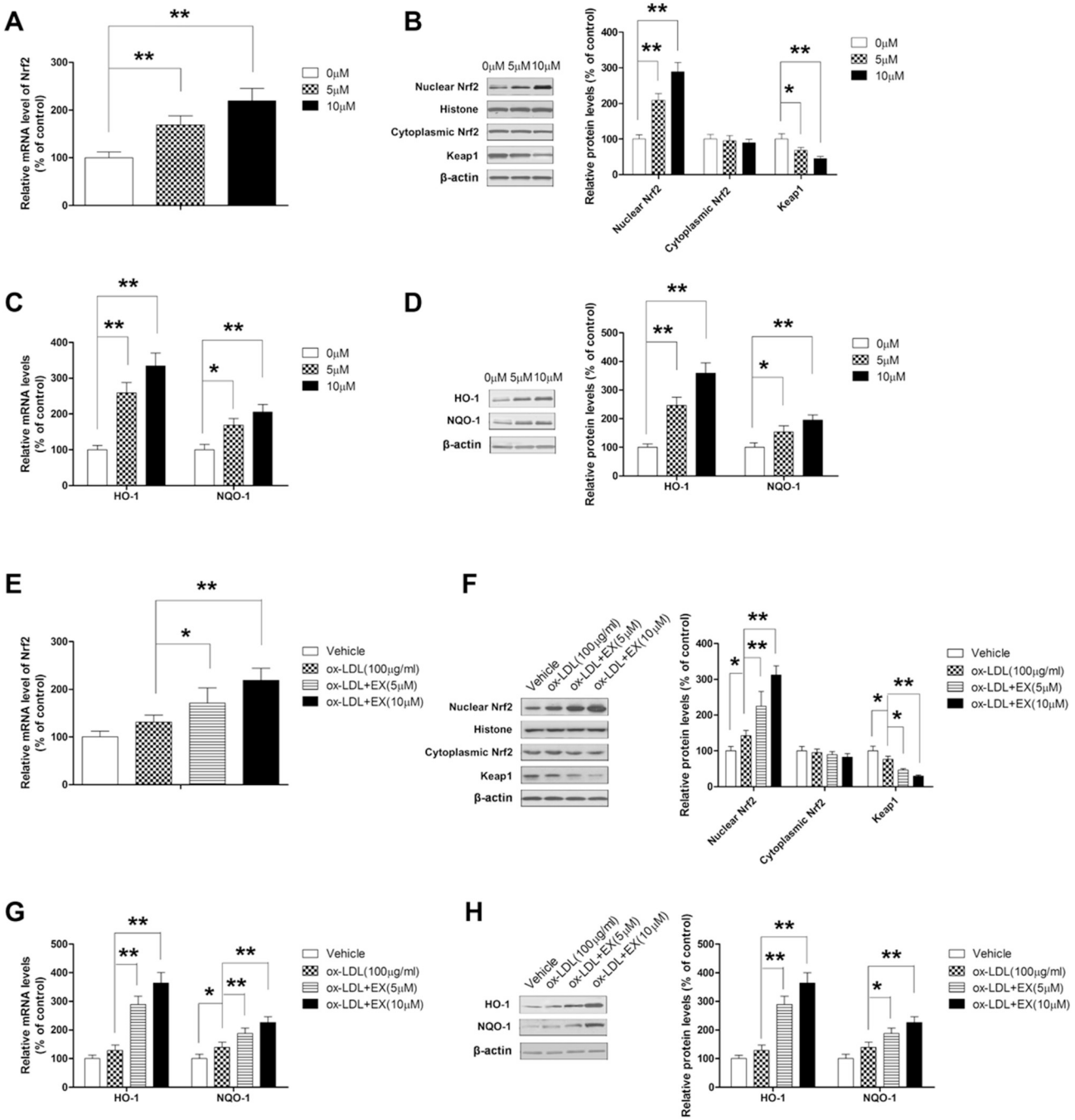

Fig. 4. Euxanthone upregulates Nrf2 and promotes its nuclear translocation in ox-LDL exposed HUVECs. (A) Euxanthone increased Nrf2 mRNA levels in HUVECs in a dose-dependent manner. (B) Euxanthone increased the expression of nuclear Nrf2 protein and downregulated Keap1 in HUVECs in a dose-dependent manner. Euxanthone upregulated HO-1 and NQO-1 mRNA (C) and proteins (D) in HUVECs in a dose-dependent manner. Euxanthone upregulated Nrf2 mRNA (E) and protein (F) levels and downregulated Keap1 (F) in HUVECs treated with ox-LDL. Euxanthone up-regulated HO-1 and NQO-1 mRNA (G) and protein (H) levels in ox-LDL-treated HUVECs. *P < 0.05, **P < 0.01.

effects of euxanthone on Nrf2 was determined using the specific ERK inhibitor PD98059. As shown in Fig. 8B and C, PD98059 significantly compromised euxanthone-induced Nrf2 upregulation and nuclear translocation. Furthermore, PD98059 significantly diminished the cyto-protective, anti-apoptotic, anti-oxidant and anti-inflammatory effects of euxanthone on ox-LDL-treated HUVECs (Fig. 8D–G). Taken together, the cyto-protective effects of euxanthone are mediated via ERK signaling (Fig. 9).

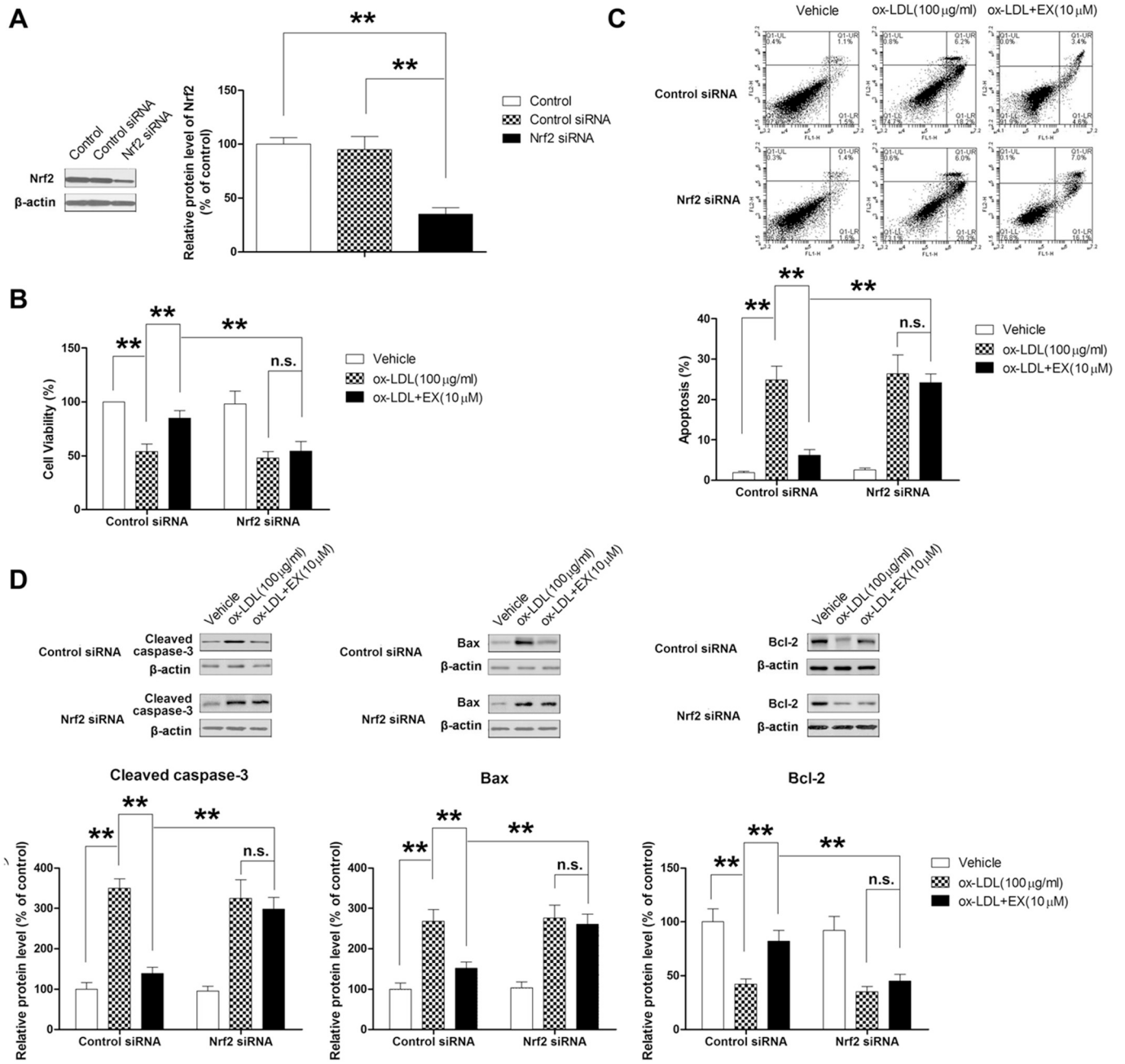

Fig. 5. Knockdown of Nrf2 markedly diminished the protective effects of euxanthone in ox-LDL-treated HUVECs. A. Immunoblot showing Nrf2 protein levels in HUVECs 48 h after Nrf2 knockdown. Euxanthone failed to protect against ox-LDL-induced cytotoxicity (B) and apoptosis (C) in HUVECs with Nrf2 knockdown. (D) Euxanthone failed to suppress cleavage of caspase-3 and PARP, up-regulate Bcl-2 and downregulate Bax, and reduce cytochrome C release in ox-LDL-treated HUVECs with Nrf2 knockdown. **P < 0.01.

4. Discussion

Since ox-LDL is closely related to the development of AS, endothelial cells (ECs) cultured with ox-LDL are used as an in vitro model for simulating AS [23]. The aim of our study was to determine potential cyto-protective effects of euxanthone, a xanthone extracted from *Polygala caudata*, against ox-LDL-injury in HUVECs, on account of its known vasodilatory effects [12,13]. Our results indicated that euxanthone protected HUVECs against ox-LDL-induced apoptosis, oxidative damage and inflammation via the Nrf2/ERK axis.

EC apoptosis, mediated by ox-LDL or other mechanisms, compromises vascular integrity, increases arterial lipid deposition, enhances vascular smooth muscle cell invasion, and stimulates monocyte migration, ultimately resulting in atherosclerotic plaque formation

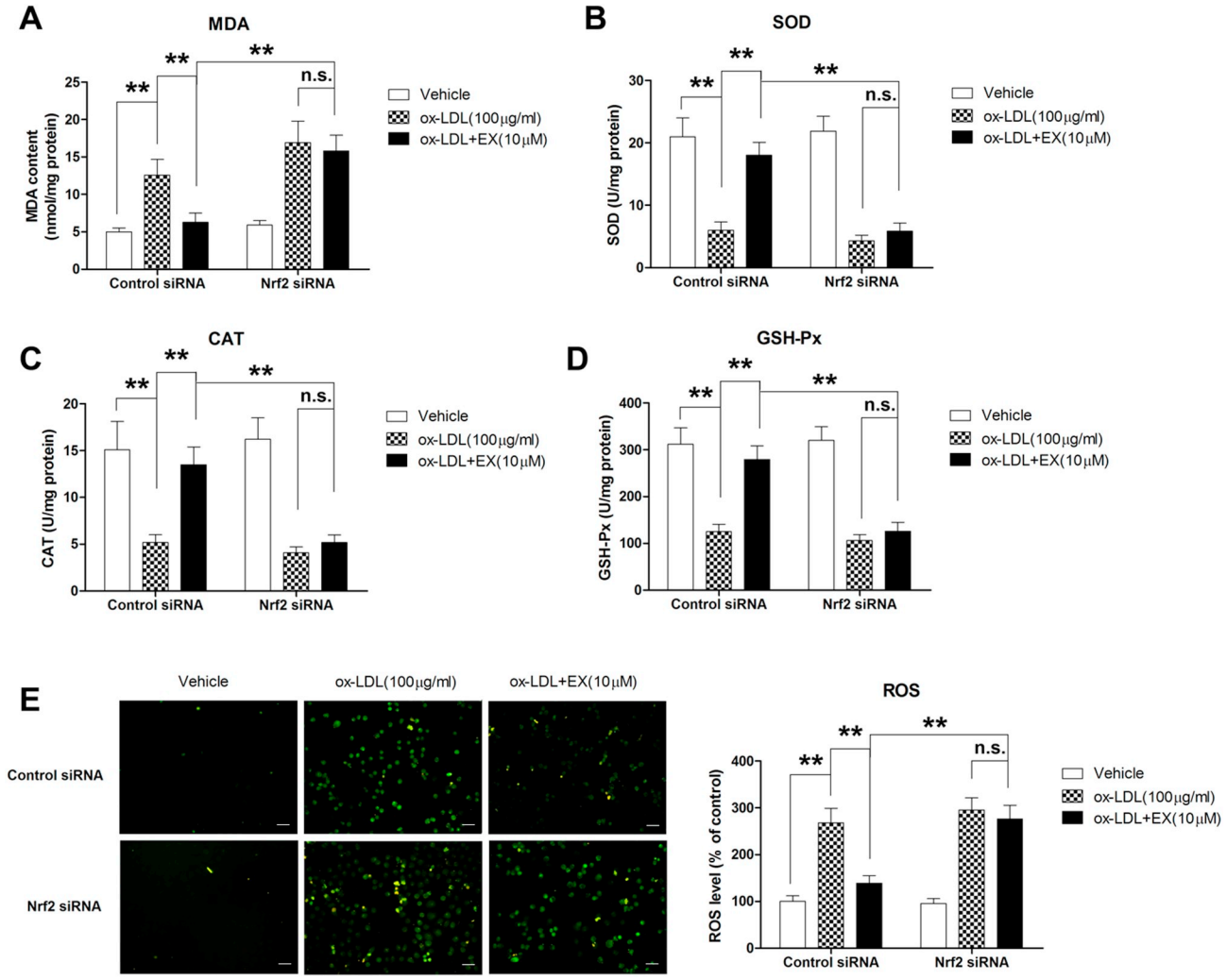

Fig. 6. Knockdown of Nrf2 markedly diminished the antioxidant effects of euxanthone in ox-LDL-treated HUVECs. Nrf2 knockdown abolished euxanthone -induced decrease in MDA levels (A), increase in the levels of SOD (B), CAT (C) and GSH-Px (D), and decrease in ROS levels in the HUVECs treated with ox-LDL. Scale bar = 200 μm. **P < 0.01.

[24,25]. Therefore, we hypothesized that abrogating the ox-LDL-induced apoptotic signaling in EC will prevent the development of AS. Ox-LDL interacts with endothelial cells via LOX-1, which decreases the MMP and opens the mitochondrial permeability transition pores (mPTP) [26]. Sustained opening of the mPTPs further depolarizes the mitochondrial membrane and releases cytochrome C into the cytoplasm, thereby triggering the apoptotic cascade. Consistent with previous studies, we found that ox-LDL promoted apoptosis in the HUVECs, as demonstrated by the increased levels of cleaved caspase-3 and PARP, reduction in MMP, and loss of cytochrome C from the mitochondria to cytosol. In contrast, pre-treatment with euxanthone markedly decreased the levels of the apoptotic factors and prevented mitochondrial dysfunction. The mitochondrial membrane protein Bcl-2 forms a complex with Bax to block the intrinsic apoptotic pathway [27]. While ox-LDL down-regulated Bcl-2 and up-regulated Bax, euxanthone reversed these changes. Taken together, euxanthone inhibited ox-LDL-induced apoptosis in the HUVECs.

Furthermore, accumulating evidence demonstrates that numerous natural products exhibit selective toxicity on tumor cells and normal cells in vitro. For example, Gao et al. reports that hispidulin could selectively kill renal cell carcinoma cells without causing obvious toxicity to normal renal tubule epithelial cells [28]. Similarly, combined our results with the prior findings, we found that euxanthone could effectively inhibit tumor cells growth as well protect HUEVCs from ox-LDL-induced cytotoxicity and apoptosis. However, the exact mechanism that contributes the distinct pharmacological effects of euxanthone against tumor cells or HUEVCs is still unclear. We postulate that this phenomenon can be explained by the tumor heterogeneity. It has been well established that tumor cells acquire distinctive and complementary capabilities that enable tumor growth and metastatic dissemination [29]. Above mentioned compounds may directly target these aberrant activation pathways and then cause toxicity effects. While in normal cells, it exhibits protective role against endogenous and exogenous stimulis. Detailed mechanisms for the effect of euxanthone on different

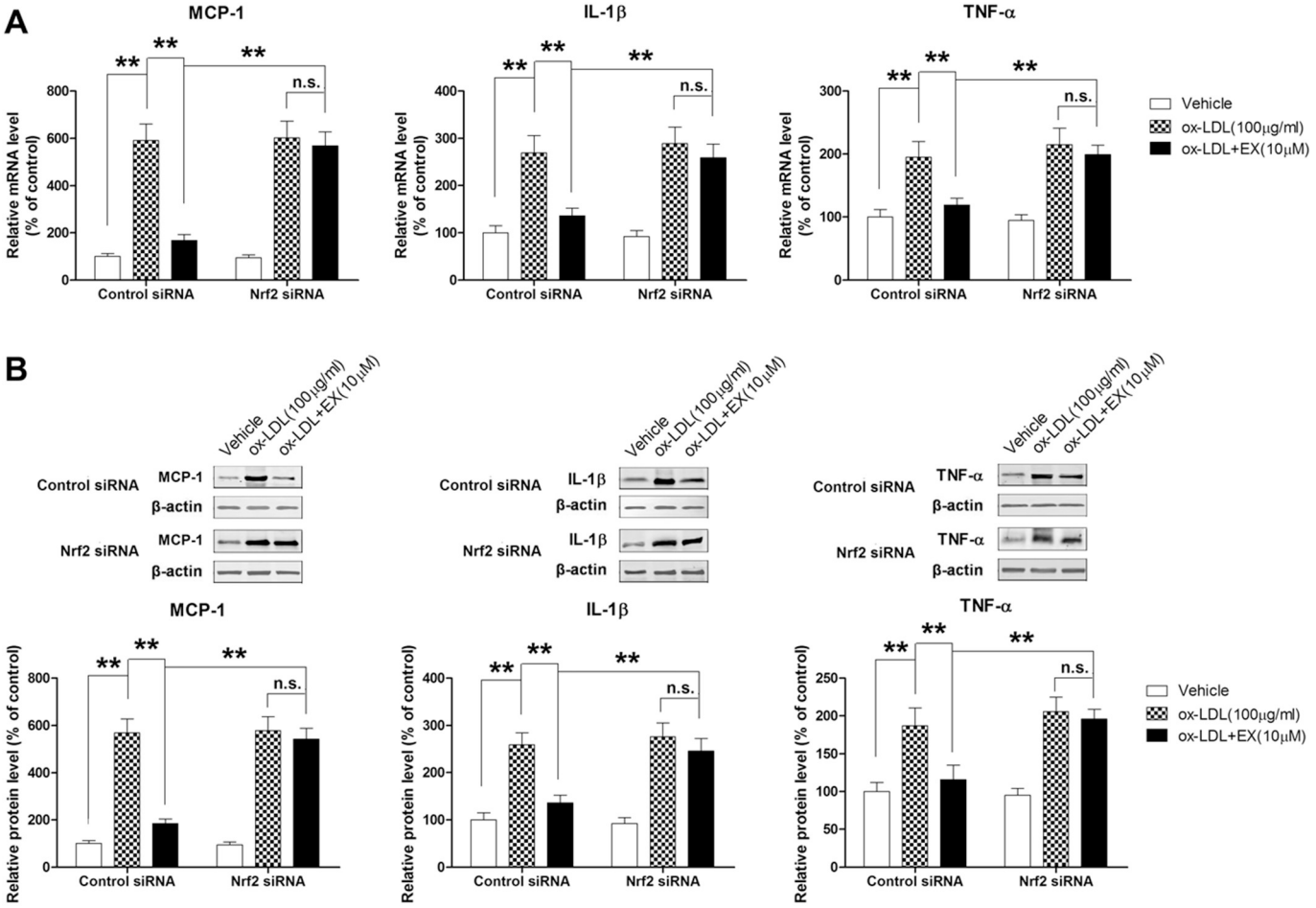

Fig. 7. Knockdown of Nrf2 markedly reduced the anti-inflammatory effects of euxanthone in ox-LDL-treated HUVECs. Nrf2 Knockdown reversed the inhibitory effect of euxanthone on MCP-1 (A, D), IL-1β (B, E) and TNF-α (C, F). **P < 0.01.

type cells will be further explored in future studies.

Oxidative stress drives AS pathogenesis through various mechanisms, including endothelial injury [30]. It is a result of excessive ROS production which overwhelms the endogenous antioxidant system, resulting in an imbalance between the free radicals and antioxidants [31]. While ox-LDL significantly increased the intracellular content of MDA in the HUVECs, and decreased that of SOD, CAT and GSH-Px, euxanthone pre-treatment activated the cellular antioxidant defense mechanism and significantly decreased the ROS levels in HUVECs. Taken together, euxanthone mitigated the oxidative stress induced by ox-LDL by increasing antioxidant enzyme activity, as well as reducing ROS production.

Inflammation is one of the major pathophysiological features of AS [32,33]. Ox-LDL triggers the secretion of inflammatory factors and endothelial cell adhesion molecules, thereby promoting monocyte recruitment to the vascular endothelium and initiating AS [34]. Therefore, anti-inflammatory drugs have also been considered to prevent and alleviate the symptoms of AS [35]. In this study also, euxanthone significantly attenuated the inflammatory response initiated by ox-LDL in HUVECs, in terms of a significant down-regulation in the levels of the pro-inflammatory cytokines IL-1β and TNF-α, and the chemokine MCP-1 that promotes monocyte recruitment and accumulation at the endothelium [36].

As a redox-sensitive transcription factor, Nrf2 plays a significant role in regulating the antioxidant defense system in various diseases, including AS [37]. Knocking out Nrf2 in the LDL receptor-knockout mice accelerated the development of atherosclerotic lesions [38]. Furthermore, Nrf2 activation inhibited inflammation in endothelial cells by directly blocking the transcription of pro-inflammatory cytokine genes [39], as well as MCP-1 secretion induced by TNF-α, NF-κB and VCAM-1 [40]. Nrf2 also regulates genes involved in lipid metabolism, apoptosis, and autophagy [41]. Several plant-derived natural compounds, including curcumin [42], miltirone [43] and zedoarondiol [7], have been shown to delay the development of AS by activating Nrf2. In line with these studies, we found that pre-treatment of the HUVECs with euxanthone activated Nrf2, while Nrf2 knockdown abolished the protective effects of euxanthone, indicating its crucial role in the cyto-protective effects of euxanthone against ox-LDL.

In conclusion, euxanthone protected HUVECs against ox-LDL-induced apoptosis, inflammation and oxidative damage by activating Nrf2 via the MAPK pathway. Therefore, euxanthone is a promising therapeutic agent against AS.

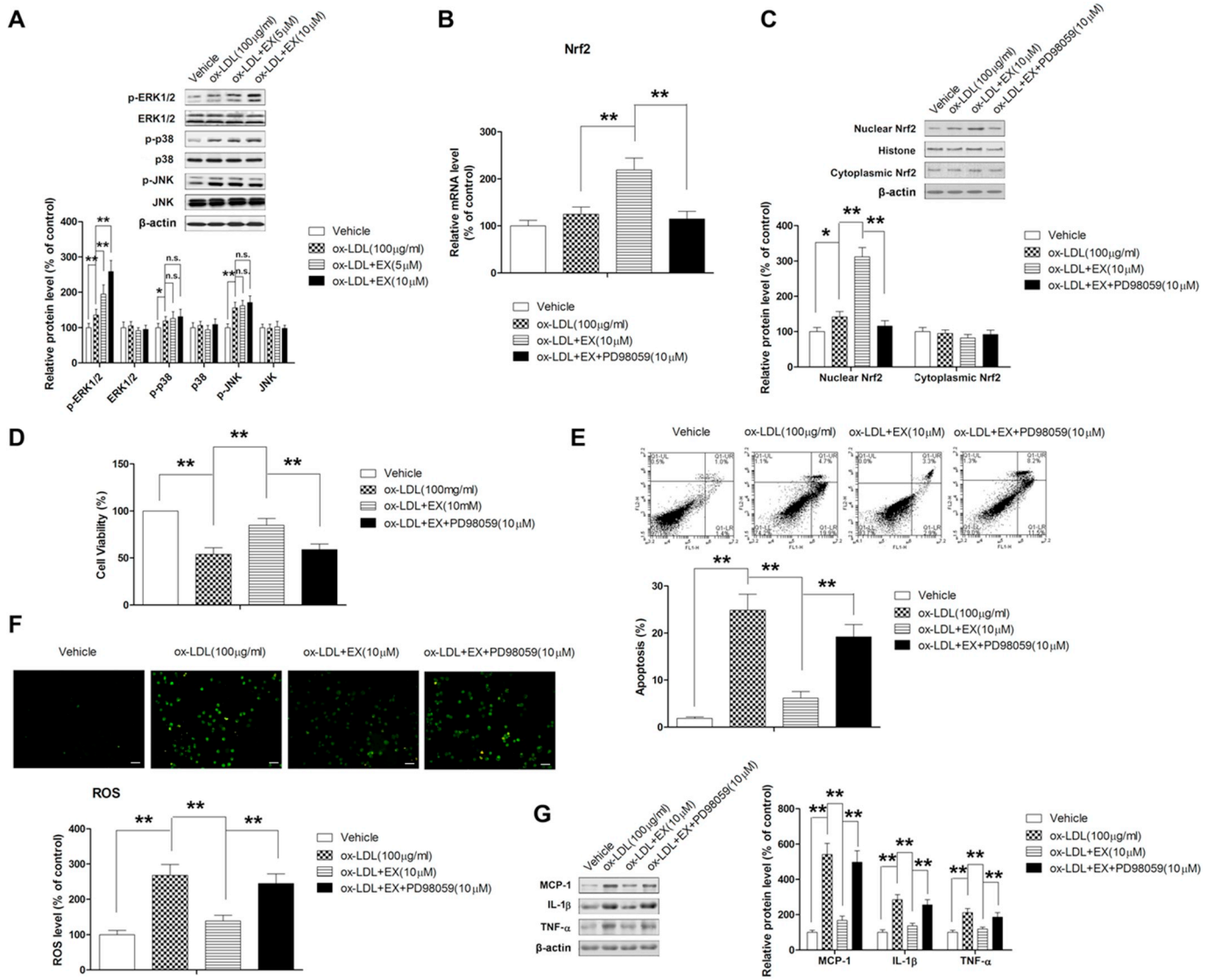

Fig. 8. Euxanthone activates Nrf2 via ERK. (A) Euxanthone activated ERK1/2 in ox-LDL-treated HUVECs. ERK1/1 inhibitor PD98059 abrogated euxanthone -induced Nrf2 upregulation (B) and nuclear translocation (C), increase in cell viability (D), inhibition of apoptosis (E), antioxidant effects (F), and anti-inflammatory effects (G) in ox-LDL-treated HUVECs. Scale bar = 200 μm. **P < 0.01.

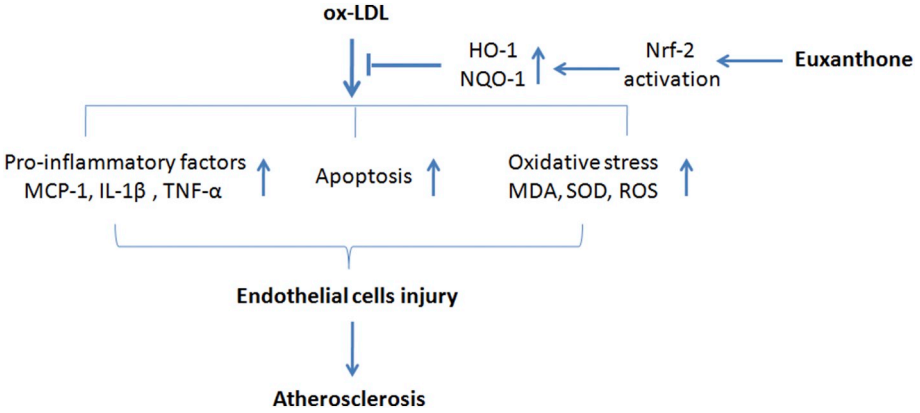

Fig. 9. Schematic diagram of the cytoprotective effects of euxanthone on ox-LDL-induced endothelial cell injury and its underlying mechanism.

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Conflict of interest

None.

Author contribution

Yong Sun designed the research and provided the fund; Shengnan Li, Zhiwu Han, Xiaocui Bu and Weijie Yu performed experiments; Shengnan Li analyzed data and wrote the paper.

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