

Tyrosol attenuates lipopolysaccharide-induced inflammation in HUVECs to promote vascular health against atherosclerosis challenge

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Abstract. The role of vascular endothelial cells in acute and chronic vascular inflammatory response has long been recognized. Therefore, persistent vascular inflammation may lead to endothelial dysfunction, thus resulting in the release of pro-inflammatory cytokines and the expression of adhesion molecules, which in turn promote monocyte/macrophage adhesion. Inflammation serves a key role in the development of vascular diseases, such as atherosclerosis. Tyrosol is a natural polyphenolic compound with diverse biological functions, found in large quantities in olive oil or in *Rhodiola rosea*. The current study aimed to investigate the regulatory *in vitro* effects of tyrosol on pro-inflammatory phenotypes using Cell Counting Kit-8, cell adhesion assay, wound healing, ELISA, western blotting, dual-luciferase, reverse transcription-quantitative PCR and flow cytometry. The results showed that tyrosol significantly inhibited the adhesion of THP-1 human umbilical vein endothelial cells, reduced lipopolysaccharide-induced cell migration and decreased the release of pro-inflammatory factors and the expression levels of adhesion-related molecules, such as TNF- α , monocyte chemotactic protein-1, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. Previous studies indicate that NF- κ B could serve a pivotal role in initiating the inflammatory responses of endothelial cells and particularly in regulating the expression of adhesion molecules and inflammatory factors. The results of the current study demonstrated that tyrosol was associated with decreased

expression of adhesion molecules and monocyte-endothelial cell adhesion, thus suggesting that tyrosol could be a novel pharmacological approach for treating inflammatory vascular diseases.

Introduction

Cardiovascular diseases cause millions of mortalities each year and remain the major causes of increased mortality worldwide. Atherosclerosis is the leading cause of the majority of cardiovascular diseases (1). Inflammation plays a key role in the pathogenesis of vascular diseases, including atherosclerosis (2). Persistent vascular inflammation may lead to endothelial dysfunction and abnormal expression of cell adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). In turn, the above effects can promote the adhesion of mononuclear macrophages to vascular endothelial cells, which is considered as a significant manifestation of early damage in cardiovascular diseases, such as atherosclerosis (3,4). Endothelial dysfunction, macrophage recruitment and inflammatory cytokine circuits are critical steps in the development of atherosclerotic diseases. Emerging evidence has suggested that anti-inflammatory factors can prevent vascular dysfunction and cardiovascular events (5,6). Lipopolysaccharide (LPS), a common surface component of Gram-negative bacteria, is an effective initiator of inflammatory responses. LPS plays a crucial role in numerous inflammatory diseases via activating the NF- κ B signaling pathway, eventually leading to the release of excessive inflammatory mediators from vascular endothelial cells. In addition, LPS is considered to be a pivotal risk factor for endothelial dysfunction (7-9). Therefore, identifying novel drugs to inhibit LPS-induced inflammation-related signaling pathways could improve the treatment of atherosclerosis.

Epidemiology has shown that the Mediterranean diet is associated with a reduced incidence of cardiovascular diseases (10). Extra virgin olive oil (EVOO), a nearly exclusive dietary fat in the Mediterranean diet, is associated with general fitness benefits and a decreased incidence of atherosclerotic diseases (11). Tyrosol [2-(4-hydroxyphenyl)-ethanol], is the most abundant biophenol in EVOO and has a broad range of

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biological effects, including antioxidant, antiapoptotic and anti-inflammatory effects (12). In addition, tyrosol is also the main active component of *Rhodiola rosea*, a plant that has been extensively used in traditional Chinese medicine (13). A previous study showed that tyrosol could exert salutary effects on inflammatory lung diseases via inhibiting inflammatory responses and maintaining the alveolar capillary barrier (14). Although, current studies have reported the enhanced anti-inflammatory activity of tyrosol, its anti-inflammatory effects and mechanisms in umbilical vein endothelial cells (HUVECs) remain elusive. Therefore, in the present study an *in vitro* inflammation model was established in LPS-stimulated HUVECs to uncover the underlying molecular mechanisms of tyrosol in atherosclerosis.

Materials and methods

Cell culture. Primary HUVECs and Tohoku Hospital Pediatrics-1 (THP-1) cells were purchased from Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd. HUVECs were cultured in Endothelial Cell Medium (cat. no. 1001; ScienCell Research Laboratories, Inc.), according to the supplier's instructions. HUVECs between passages 4 and 6 were used for the experiments. THP-1 cells were grown in PRMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 1% penicillin/streptomycin solution and 10% FBS (Gibco; Thermo Fisher Scientific, Inc.).

Cell viability assay. Cell viability was assessed using a Cell Counting Kit 8 (CCK-8) assay (Dojindo Laboratories, Inc.), according to the manufacturer's instructions. Briefly, HUVECs were cultured in 96-well plates overnight and were then treated with different concentrations of tyrosol (0.3, 0.5, 0.7, 1, 1.2, 1.5 and 2 mmol/l) at 37°C for 24 h. Subsequently, culture medium supplemented with 10 % CCK-8 reagent was added into each well and cells were cultured at 37°C for an additional 1.5 h. The absorbance at a wavelength of 450 nm was measured using an enzyme labelling apparatus.

Cell adhesion assay. Cell adhesion assay was performed as previously described (15). Briefly, HUVECs were seeded into 12-well plates and cultured until reached ~90% confluence. Prior to stimulation with LPS, cells were treated with tyrosol (0.5 mM or 1 mM) at 37°C for 4 h. THP-1 cells were then labelled with the fluorescent pH indicator, BCECF-AM (5 μ M; cat. no. S1006; Beyotime Institute of Biotechnology), for 30 min and were allowed to adhere on the endothelial cell monolayer at 37°C for 60 min. Following washing with PBS, residuary adherent THP-1 cells were analyzed with a fluorimeter (Tecan Group, Ltd.).

HUVECs wound healing assay. HUVECs at a density of 5×10^5 cells/well were cultured in 6-well plates and grown until they reached 90% confluence. The HUVECs monolayer was then scratched with a 200- μ l pipette tip, followed by rinsing with PBS and cell treatment with LPS (100 ng/ml) or tyrosol (0.5 mM or 1 mM) at 37°C for 12 h. HUVECs were incubated in FBS-free Endothelial Cell Medium (cat. no. 1001; ScienCell Research Laboratories, Inc.) for 12 h and the cell migration ability was assessed under an inverted microscope (Olympus

Corporation) in three randomly selected fields. Migratory cells were analyzed using ImageJ software (version 1.5; National Institutes of Health).

ELISA. The secretion levels of TNF- α and monocyte chemotactic protein-1 (MCP-1) were measured using the corresponding Human TNF- α ELISA kit (cat. no. EK0525) and Human MCP-1 ELISA kit (cat. no. EK0441), according to the manufacturer's protocol (Wuhan Boster Biological Technology, Ltd.).

Western blot analysis. For western blot analysis, total proteins were extracted from HUVECs using RIPA Lysis and Extraction Buffer (cat. no. 89900; Thermo Fisher Scientific, Inc.). The proteins were then centrifuged at 14,000 \times g, for 15 min at 4°C and the protein concentration was measured using a BCA protein assay kit (Beyotime Institute of Biotechnology). Subsequently, 10-30 μ g total protein/sample was separated by 10% SDS-PAGE, followed by transfer to PVDF membranes (cat. no. IPVH00010; MilliporeSigma). Following blocking with 5% BSA for 1 h at room temperature, the membranes were incubated with primary antibodies in a 4°C refrigerator overnight. The membranes were then washed thrice with TBS-1% Tween-20 for 10 min each, followed by incubation with the corresponding secondary antibodies at room temperature for 2 h. The antibodies used were as follows: Mouse anti-ICAM-1 (dilution, 1:1,000; cat. no. ab20), rabbit anti-VCAM-1 (dilution, 1:1,000; cat. no. ab181315), rabbit GAPDH (dilution, 1:1,000; cat. no. ab181602; all from Abcam), rabbit anti-phosphorylated(p)-p65 (dilution, 1:1,000; cat. no. 3033S), rabbit anti-p65 (dilution, 1:1,000; cat. no. 8242T), rabbit anti-p-I κ B α (dilution, 1:1,000; cat. no. 2859T), mouse anti-I κ B α (dilution, 1:1,000; cat. no. 4814S) (all from CST Biological Reagents Co., Ltd.) and horseradish peroxidase-coupled secondary antibodies (dilution, 1:10,000; cat. nos. ab6728 and ab6721; Abcam). Following detection with an ECL reagent (Shanghai Yeasen Biotechnology, Co., Ltd.), ImageJ software (version 1.5; National Institutes of Health) was used for densitometric analysis. The relative expression levels of the target proteins were normalized to those of GAPDH.

Dual luciferase reporter assay. HUVECs at a density of 10^4 cells/well were seeded into 96-well plates and were then co-transfected with 100 ng NF- κ B luciferase reporter plasmid (cat. no. YB003B; Shanghai Yu Bo Biological Technology Co., Ltd.) and 10 ng pGMLR-CMV luciferase Reporter plasmid (cat. no. 11558ES03; Shanghai Yeasen Biotechnology, Co., Ltd.) using Lipofectamine[®] 3000 (cat. no. L3000015; Invitrogen; Thermo Fisher Scientific, Inc.). At 24 h post-transfection, cells were stimulated with 100 ng/ml LPS with or without tyrosol (0.5 mM or 1 mM) at 37°C for 24 h. Luciferase activity was measured using the corresponding Dual-Luciferase Assay kit (Shanghai Yeasen Biotechnology, Co., Ltd.). *Renilla* luciferase was used as internal reference.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from HUVECs at a density of 5×10^5 cells/well using an RNA extraction kit (EZBioscience). Subsequently, ~1 μ g of total RNA was reverse transcribed into

cDNA using the Evo M-MLV Reverse Transcription kit (cat. no. AG11705; Accurate Biology). The target genes ICAM-1, VCAM-1 and the internal reference gene GAPDH were amplified by qPCR using the 2X SYBR[®] Green Pro Taq HS Premix (cat. no. AG11718; Accurate Biology) kit. The thermocycling conditions used were as follows: For gDNA removal: 2 min at 42°C, followed by keeping at 4°C; for reverse transcription PCR: 15 min at 37°C and 5 sec at 85°C, followed by keeping at 4°C; and for qPCR: 95°C for 30 sec, followed by 40 cycles at 95°C for 5 sec and 60°C for 30 sec, followed by dissociation stage. All of the above steps were performed according to the manufacturer's protocols. The primer sequences used were as follows: VCAM-1, forward, 5'-GAGATACAACCGTCTTGG-3' and reverse, 5'-CCTTCACATAAATAAACC-3'; ICAM-1, forward, 5'-ATGGCAACGACTCCTTCTC-3' and reverse, 5'-TGTCACCTCGGTCCCTTC-3'; and GAPDH, forward, 5'-TCTGACTTCAACAGCGACACC-3' and reverse, 5'-CTGTTGCTGTAGCCAAATTCGTT-3' (Generay Biotech Co., Ltd.). The amplification data were quantified using the 2^{-ΔΔC_q} method (16) and the relative expression levels of target genes were then calculated. These experiments were replicated in triplicate.

Flow cytometry (FCM) analysis. To promote the differentiation of THP-1 cells into macrophages, cells were seeded into 6-well plates and were then induced with 0.1 μg/ml phorbol myristate acetate (PMA) for at 37°C 72 h. The suspended cells were washed away using PBS. Following treatment of PMA-derived THP-1 cells with tyrosol at 37°C for 4 h, cells were then stimulated with 100 ng/ml LPS at 37°C for 24 h, followed by washing twice with staining buffer (BioLegend, Inc.). Subsequently, cells were incubated with anti-human/mouse FITC-CD86 at room temperature for 20 min. After incubation, cells were collected, washed and resuspended in cell staining buffer. The percentage of CD86 positive cells was measured using FCM (CytExpert; Beckman Coulter, Inc.) and the results were analyzed using FlowJo v10.0.6 software (FlowJo LLC).

Statistical analysis. Each experiment was performed in triplicate and data analysis and output were performed using GraphPad Prism 7 (GraphPad Software; Dotmatics) software. All data are expressed as the mean ± standard error of the mean. The differences among multiple groups were analyzed by one-way ANOVA analysis followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Tyrosol inhibits LPS-induced monocyte-endothelial cell adhesion. No statistically significant difference was observed in the viability of HUVECs treated with 0-2 mM tyrosol (Fig. 1B). Eventually, the concentrations of 0.5 and 1 mM were selected for the following experiments. It has been reported that the induction of pro-inflammatory cytokines and adhesion molecules during vascular inflammation can promote the adhesion and infiltration of monocytes into the endothelium, thus resulting in the occurrence of atherosclerosis (4). To assess the effect of tyrosol on monocyte-endothelial cell interactions, the adhesion of

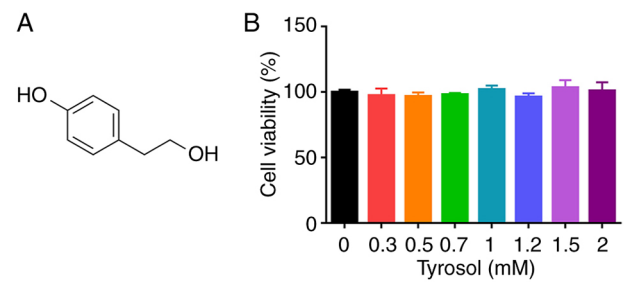


Figure 1. (A) Molecular structure of tyrosol. (B) Effect of tyrosol on cell viability after treatment with the indicated concentrations of tyrosol (0.3, 0.5, 0.7, 1, 1.2, 1.5 and 2 mmol/l) for 24 h, cell viability was measured and expressed as mean ± standard error of the mean for three independent experiments.

monocytes to HUVECs was evaluated by fluorescence labelling (Fig. 2A). As shown in Fig. 2B, when HUVECs were treated with 100 ng/ml LPS, the number of THP-1 cells adhering to HUVECs were obviously increased to approximately 4.5-fold compared with the control group. However, HUVECs pre-treated with tyrosol significantly declined LPS-mediated enhanced cell adhesion. The above results suggested that tyrosol could constrain the adhesion of THP-1 cells to LPS-induced HUVECs.

Tyrosol attenuates LPS-induced HUVECs migration. Emerging evidence has suggested that LPS, an inflammatory mediator, induces HUVECs inflammation *in vitro* (7-9). To investigate the effect of tyrosol on cell migration, the HUVECs migration ability was evaluated using wound healing assay. Following cell induction with LPS for 12 h, the migration ability of HUVECs was markedly enhanced (Fig. 2C). Following cell pre-treatment with tyrosol (0.5 and 1 mM) for 12 h, the number of migrated cells was reduced and the scratch area without cells was markedly increased compared with cells treated with LPS alone (Fig. 2D). The above findings indicated that tyrosol could restrain LPS-induced HUVECs migration.

Tyrosol mitigates the LPS-induced expression of adhesion molecules in HUVECs. Subsequently, the expression levels of adhesion-related molecules were detected in HUVECs to determine whether tyrosol could exert a repressive effect on LPS-induced endothelial cell inflammation. The protein expression levels of ICAM-1 and VCAM-1 were also markedly decreased in tyrosol-treated LPS-induced HUVECs (Fig. 3A and B). Consistent with the western blotting results, the relative mRNA expression levels of ICAM-1 and VCAM-1 were also notably increased in LPS-treated HUVECs (Fig. 3C). However, cell pre-treatment with tyrosol abrogated the effect of LPS on the mRNA expression levels of the above genes.

Tyrosol ameliorates the LPS-induced expression of pro-inflammatory cytokines. It has been reported that the excessive secretion of cytokines and chemokines is closely associated with endothelial dysfunction (3). In the present study, the contents of TNF-α and MCP-1 in the culture supernatant were significantly enhanced by ~1.7- and 2.4-fold, respectively, in

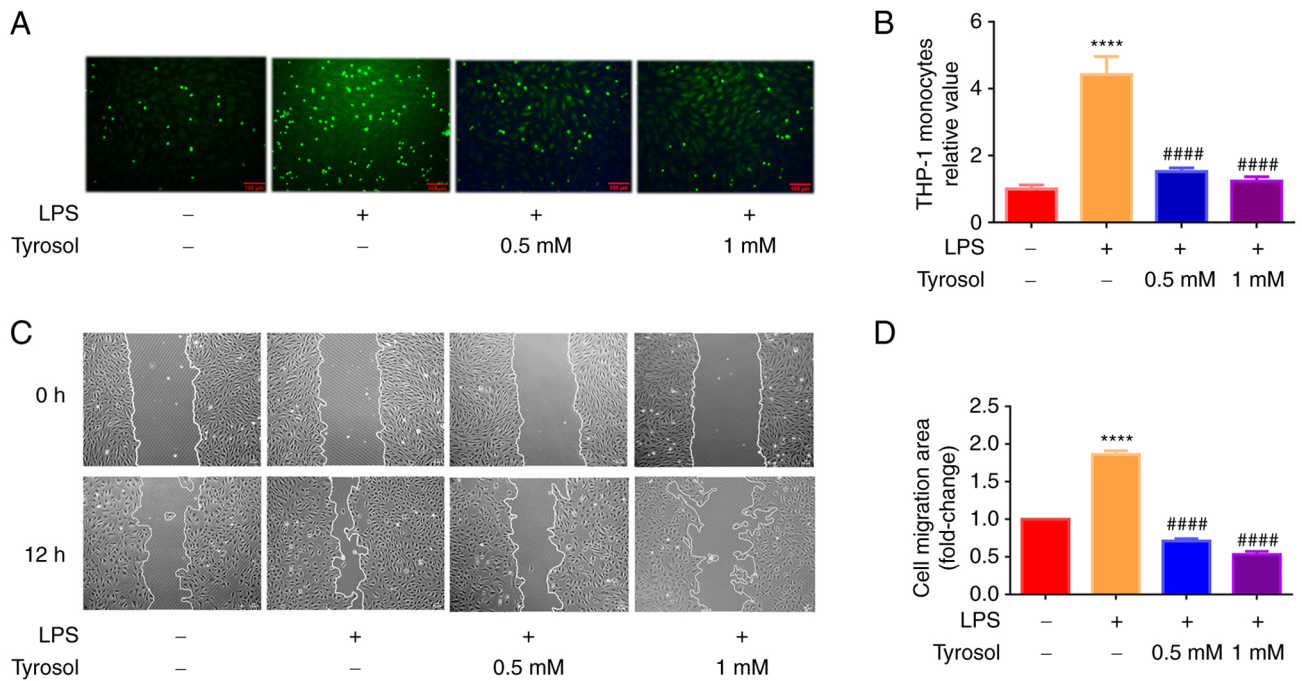


Figure 2. Tyrosol attenuates LPS-induced attachment of THP-1 monocytes to HUVECs and migration of HUVECs in the cell scratch test. HUVECs were incubated tyrosol (0.5 and 1 mM) for 4 h and then stimulated with LPS (100 ng/ml) for 24 h. (A) Representative images used for quantification of the fold changes in THP-1 cells attached to HUVECs. Scale bar, 100 μ m. (B) Quantification of the fold changes in adhesion. The attachment of THP-1 monocytes to HUVECs was determined. (C) Migration of HUVECs was tested by wound healing assay. Scale bar, 50 μ m. (D) Wound healing was quantified by measuring the migrated area of HUVECs. Data represent the mean \pm standard error of the mean (**** P <0.0001 vs. control; #### P <0.0001 vs. treatment with LPS alone). LPS, lipopolysaccharide; HUVECs, umbilical vein endothelial cells.

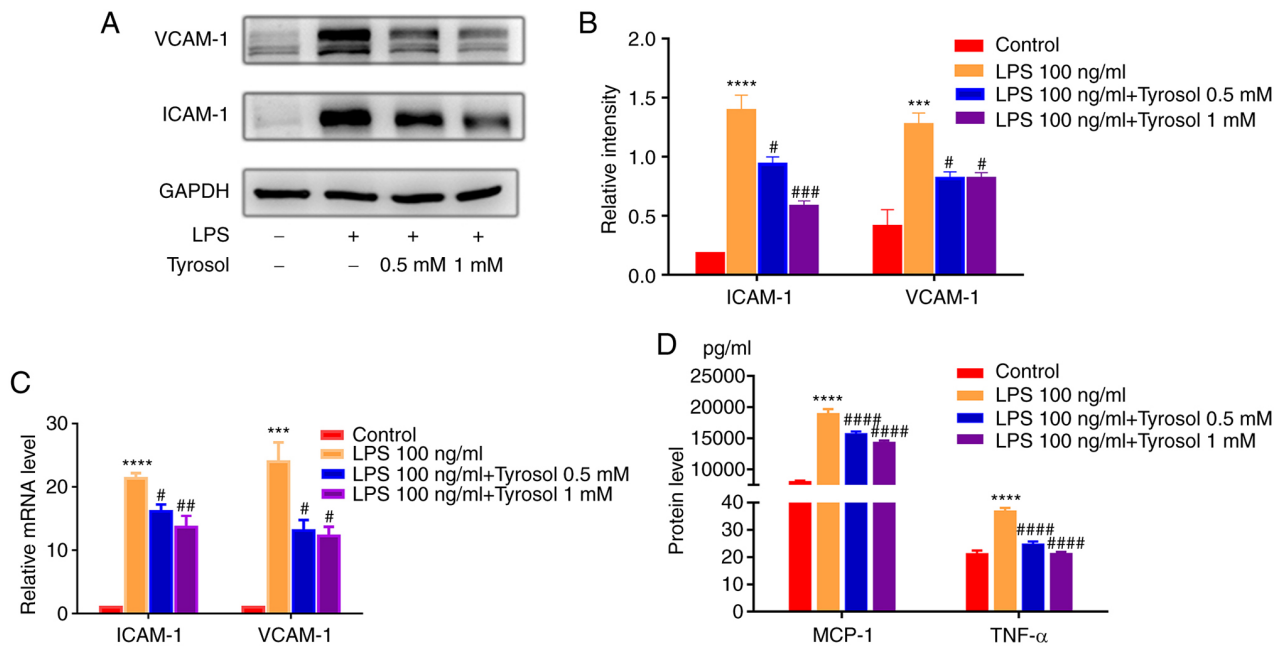


Figure 3. Tyrosol decreases the expression of adhesion molecules and inflammatory factors in HUVECs. HUVECs were incubated with tyrosol for 4 h and then stimulated with LPS (100 ng/ml) for 24 h. (A) Western blot analysis. (B) Quantification of the fold changes in ICAM-1 and VCAM-1 expression in HUVECs. (C) Reverse transcription-quantitative PCR analysis of ICAM-1 and VCAM-1 expression. (D) Expression of TNF- α and MCP-1 detected by ELISA. Data represent the mean \pm standard error of the mean (**** P <0.0001 and **** P <0.0001 vs. control; # P <0.05, ## P <0.01, ### P <0.001 and ##### P <0.0001 vs. treatment with LPS alone). HUVECs, umbilical vein endothelial cells; LPS, lipopolysaccharide; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; MCP-1, monocyte chemoattractant protein-1.

LPS-stimulated HUVECs. Notably, the LPS-induced secretion of TNF- α and MCP-1 were markedly inhibited by tyrosol treatment (Fig. 3D).

Tyrosol attenuates the polarization of LPS-induced macrophages to M1 phenotype. It has been reported that activated vascular endothelial cells can accelerate circulating

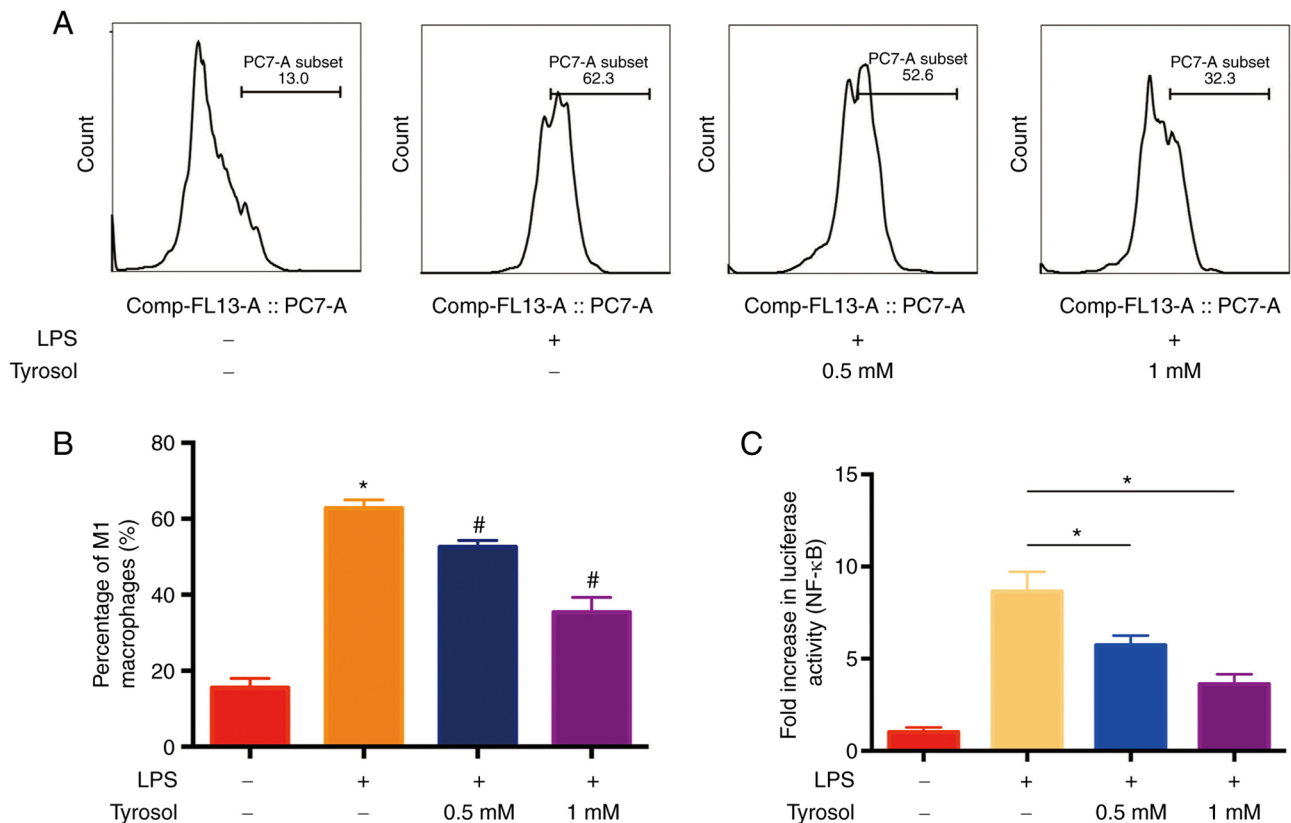


Figure 4. Tyrosol reduces LPS-induced M1 macrophages polarization and the luciferase activity of the promoter of NF- κ B. PMA-derived THP-1 cells were incubated with tyrosol for 4 h and then stimulated with LPS (100 ng/ml) for 24 h. (A) Flow cytometry analysis. (B) Percentage of CD86 of THP-1-derived macrophages. (C) Luciferase activity of NF- κ B was measured. Data represent the mean \pm standard error of the mean (* P <0.05 compared with control; # P <0.05 compared to treatment with LPS alone). LPS, lipopolysaccharide; PMA, phorbol myristate acetate.

monocytes to enter the subendothelial and differentiate into macrophages (3,4). In addition, macrophages produce abundant cytokines and exacerbate vascular inflammation. Therefore, to investigate whether tyrosol could attenuate the polarization of macrophages to M1 phenotype, the percentage of CD86 positive M1 cells was measured using FCM analysis (Fig. 4A). As shown in Fig. 4B, macrophage stimulation with LPS alone promoted their polarization to M1 phenotype. Additionally, the percentage of CD86-positive THP-1 macrophages was increased by ~60%, which was dose-dependently reduced by tyrosol.

Tyrosol suppresses the activity of NF- κ B promoter. ICAM-1 and VCAM-1 is a critical cell adhesion molecules activated by NF- κ B in HUVECs (9). The current study hypothesized that tyrosol could promote the inactivation of NF- κ B. Therefore, the effect of LPS on the activity of NF- κ B promoter was first assessed using dual luciferase activity assay. More specifically, cell treatment with LPS alone, notably augmented the luciferase activity of NF- κ B promoter by ~9-fold. However, the activity of NF- κ B promoter was diminished by ~6- and 4-folds following cell treatment with 0.5 and 1 mM tyrosol, respectively (Fig. 4C).

Tyrosol restrains the activation of NF- κ B induced by LPS. NF- κ B is considered as the principal signaling pathway involved in the regulation of inflammatory responses and the expression of inflammatory genes in endothelial cells (9).

Therefore, to explore the effects of tyrosol on the NF- κ B signaling pathway in LPS-treated HUVECs, the expression levels of p-p65, p65, p-I κ B α and I κ B α were determined using western blot analysis (Fig. 5A). No significant differences were observed in the total levels of p65 (Fig. 5B), whereas, the protein expression and phosphorylation levels of p-p65 (Fig. 5C and D) and p-I κ B α (Fig. 5E) were notably enhanced in HUVECs induced by LPS, which were restored in cells pre-treated 0.5 mM or 1 mM tyrosol. The aforementioned findings demonstrated that cell treatment with tyrosol could inhibit the LPS-induced activation of NF- κ B signaling.

Discussion

It has been reported that vascular endothelial cells serve a vital role in acute and chronic inflammatory responses caused by injury or infection (17). Persistent vascular inflammation can cause endothelial dysfunction, which in turn may result in the development of vascular diseases, such as atherosclerosis (18). The inflammatory response in local blood vessels commonly leads to atherosclerosis, which is a major underlying cause of various cardiovascular diseases. In the present study, HUVECs treatment with 0-2 mM tyrosol, isolated from EVOO or *Rhodiola rosea*, had minor effect on cell viability. Mounting evidence has suggested that monocyte adhesion, vascular endothelial cell migration and macrophage polarization exert a crucial role in the development of atherosclerosis (19). The results of the current study

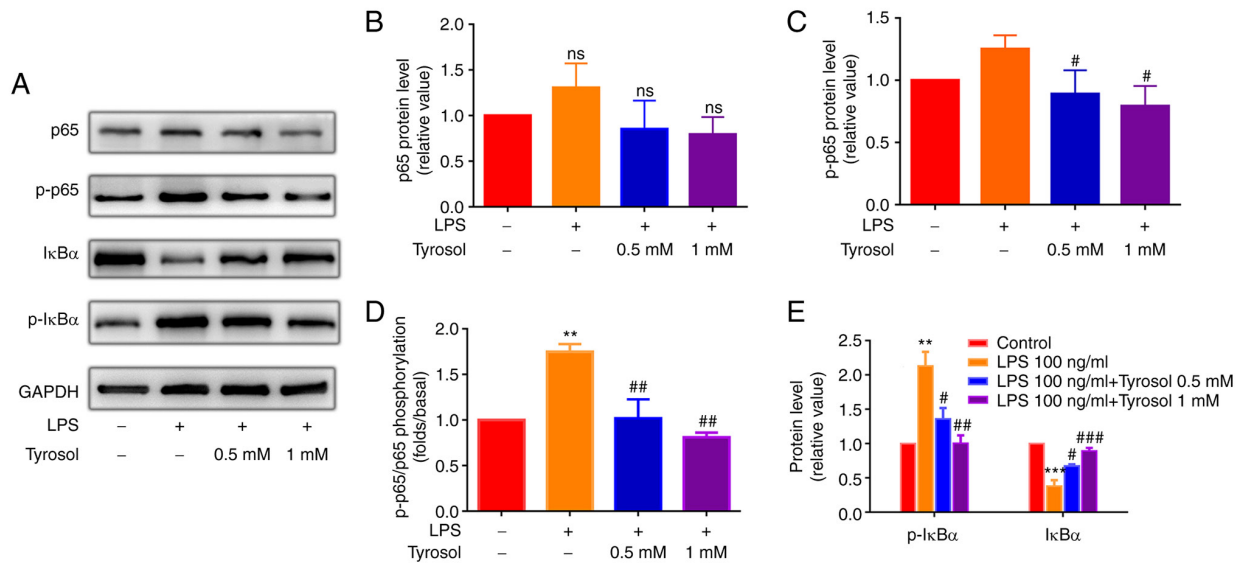


Figure 5. Tyrosol inhibits the phosphorylation of p65 and IκBα. HUVECs were incubated with tyrosol for 4 h and then stimulated with LPS (100 ng/ml) for 24 h. (A) Western blot analysis of p-p65; total p65; p-IκBα and total IκBα. B-E, Quantification of (B) p65, (C) p-p65, (D) p-p65/p65 and (E) p-IκBα and IκBα expression. Data represent the mean ± standard error of the mean (**P<0.01 and ***P<0.001 vs. control; #P<0.05, ##P<0.01 and ###P<0.001 vs. treatment with LPS alone; ns, no significance). HUVECs, umbilical vein endothelial cells; LPS, lipopolysaccharide; p-, phosphorylated.

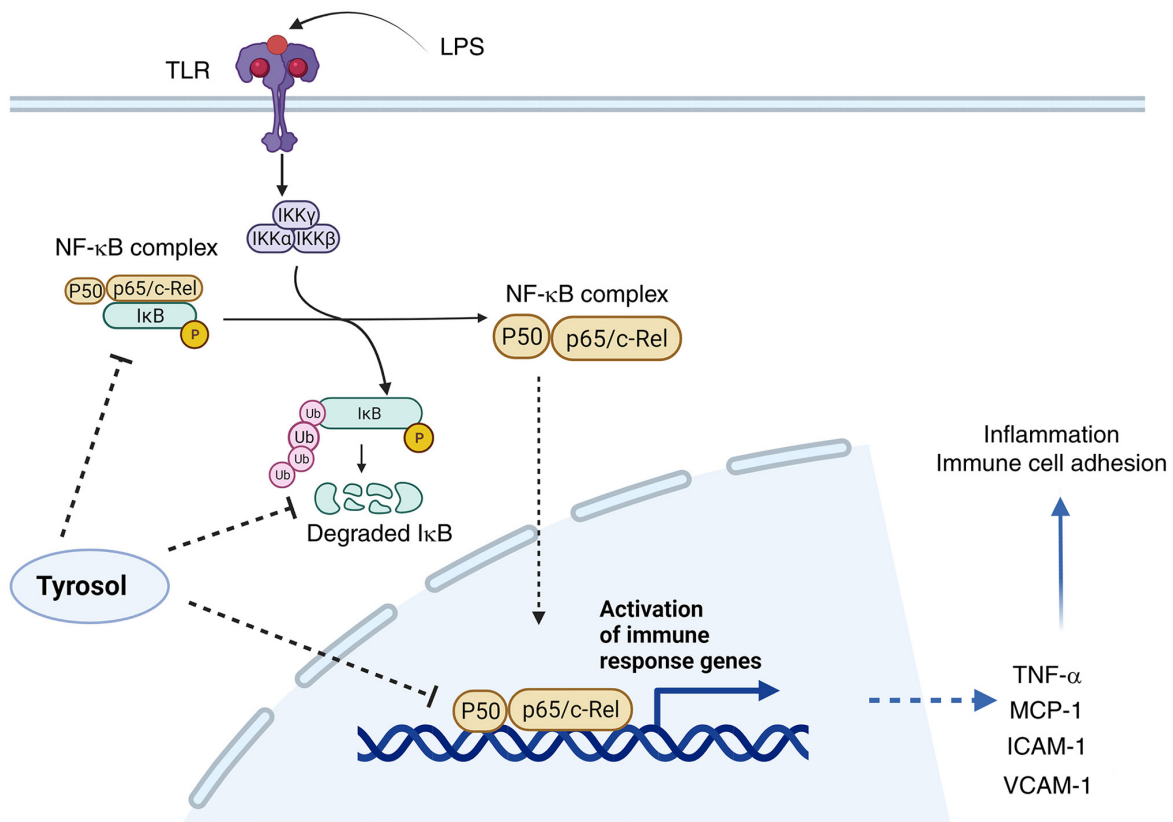


Figure 6. Schematic illustration of possible potential mechanisms for the anti-inflammatory potential of tyrosol. Tyrosol inhibited LPS-induced phosphorylation and degradation of IκBα and phosphorylation of p65, thereby attenuating inflammation in endothelial cells against atherosclerosis challenge. LPS, lipopolysaccharide; TLR, Toll-like receptor; MCP-1, monocyte chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

demonstrated that tyrosol markedly reduced the adhesion of monocytic THP-1 cells onto LPS-induced HUVECs, the migration ability of endothelial cells and the conversion of macrophages into M1 phenotype.

Interactions between cell adhesion molecules serve a critical role in activating, promoting and maintaining macrophage recruitment in the inflammatory vascular lesion zone (4). Therefore, drugs inhibiting the expression of adhesion-related

molecules and pro-inflammatory cytokines could be a potent approach for treating inflammatory vascular diseases. It has been also reported that the adhesion rate of monocytes onto endothelial cells can be affected by downregulation of VCAM-1 and ICAM-1, two major adhesion molecules (15). Previous studies (2-4) revealed that the upregulation of adhesion molecules could be associated with the formation of atherosclerosis, while inhibition of their expression could greatly attenuate aortic plaque formation. Pro-inflammatory cytokines are a momentous pathogenic factor involved in the progression of several diseases, including atherosclerosis. In addition, a previous study showed that MCP-1 could serve a significant role in atherosclerosis via recruiting immune cells to invade the arterial wall (20). The results of the present study showed that tyrosol improved THP-1 cell adhesion onto HUVECs via inhibiting the expression of adhesion-related molecules and pro-inflammatory cytokines in LPS-induced HUVECs.

Inflammatory responses are present in all stages of atherosclerosis (21). A study demonstrated that pro-inflammatory signaling networks in vascular endothelial cells could regulate the expression of adhesion molecules and leukocyte cytokines by activating NF- κ B (22). Notably, arterial endothelium at sites prone to plaque formation, such as arterial branches and non-laminar flow areas, promoted the structural activation of NF- κ B (23). Therefore, inhibition of NF- κ B signaling could prevent the development of atherosclerosis. The results of the present study revealed that tyrosol could suppress endothelial inflammation by inhibiting the NF- κ B signaling pathway. LPS activates NF- κ B signaling via Toll-like receptors (24). Therefore, once the NF- κ B signaling pathway is activated, I κ B α dissociates from NF- κ B and the two molecules are then phosphorylated (24). Upon p65 dissociation, I κ B α is phosphorylated by IKK, followed by immediate ubiquitination and degradation (25,26), thus resulting in the activation of the distinct NF- κ B signaling pathway. In the present study, western blot analysis showed that cell treatment with tyrosol significantly inhibited LPS-induced phosphorylation and subsequent degradation of I κ B α and phosphorylation of p65 in HUVECs, suggesting that tyrosol could exert a protective effect against inflammation in endothelial cells via inhibiting NF- κ B signaling. The present study also aimed to uncover the possible mechanisms underlying the effect of tyrosol on regulating p65 expression. Therefore, the activity of p65 promoter was detected in tyrosol-treated HUVECs via subcloning reporter gene elements into *Renilla* plasmid reporter constructs, which were then transfected into HUVECs. The dual luciferase assay showed that the activity of NF- κ B promoter was progressively reduced. However, further investigation is required to verify whether there are binding sites for tyrosol on NF- κ B promoter.

Collectively, the results of the current study demonstrated that tyrosol could alleviate endothelial inflammatory responses *in vitro*, which could be partially caused by inhibition of NF- κ B signaling activation. In addition, cell treatment with tyrosol notably reduced the percentage of LPS-induced M1 macrophages. The aforementioned findings could provide novel insights into the role of tyrosol as a potential therapeutic approach for treating several diseases associated with endothelial inflammation (Fig. 6). However, the present study had some limitations. The beneficial effects of tyrosol were only investigated in a LPS-stimulated endothelial cell culture model. The pathophysiology of atherosclerosis is complex

and therefore further investigations are needed to clarify its underlying molecular mechanisms.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WZ, HW and TL contributed to the design of the present study. WZ, HW, DS and TP performed the experiments and analyzed the data. JL and WS drafted the manuscript and made substantial contributions to conception and design. WZ and TL confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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