Tartary Buckwheat Protein-derived Peptide AFYRW alleviates H2O2-induced vascular injury via the PI3K/AKT/NF-κB pathway

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Research Article

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Abstract

Tartary buckwheat protein-derived peptide (Ala-Phe-Tyr-Arg-Trp, AFYRW) is a natural active peptide that hampers the atherosclerosis process, but the underlying role of AFYRW in angiogenesis remains unknown. Here, we present a system-based study to evaluate the effects of AFYRW on H\textsubscript{2}O\textsubscript{2}-induced vascular injury in human umbilical vein endothelial cells (HUVECs). HUVECs were co-incubated with H\textsubscript{2}O\textsubscript{2} for 2 h to the vascular injury model, and AFYRW was added 24 h in advance to investigate the protective mechanism of vascular injury. We identified that AFYRW inhibits oxidative stress, cell migration, cell invasion, and angiogenesis in H\textsubscript{2}O\textsubscript{2}-treated HUVECs. In addition, we found H\textsubscript{2}O\textsubscript{2}-induced upregulation of phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), phosphorylation of nuclear factor-κB (NF-κB) p65 and nuclear translocation of NF-κB decreased by AFYRW. Taken together, AFYRW attenuated H\textsubscript{2}O\textsubscript{2}-induced vascular injury through the PI3K/AKT/NF-κB pathway. Thereby, AFYRW may serve as a therapeutic option for vascular injury.

Introduction

The formation of new blood vessels, or angiogenesis, undergoes migration and proliferation of endothelial cells to develop new blood vessels from capillaries [1]. The vital steps of angiogenesis include the proliferation, migration, differentiation, and tube formation of vascular endothelial cells. Angiogenesis is a complex process that plays important roles in embryonic development, reproduction, menstrual cycle, and wound repair [1]. Aberrant angiogenesis is observed as a promoter of disease development, especially in tumor development and blindness in diabetes [1]. Despite the fact that inhibition of angiogenesis is an established treatment strategy for many diseases, the present therapies are, for the most part, far from satisfying.

Angiogenesis is usually accompanied by oxidative stress due to reactive oxygen (ROS), which promote angiogenesis by producing active oxidation products [1]. ROS not only supports angiogenesis by up-regulating vascular endothelial growth factor (VEGF), but also acts as a key regulator of angiogenesis to induce endothelial cell migration, proliferation, and tubular structure formation in vivo. It is noteworthy that ROS regulates a variety of cellular signaling pathways, such as PI3K/Akt and NF-κB [1]. The PI3K/Akt pathway is involved in proliferation, adhesion, migration, invasion, metabolism, survival, and angiogenesis [1]. PI3K, intracellular phosphatidylinositol kinase, is activated by dimer conformational changes caused by upstream signal factors, and the activated PI3K then drives AKT. AKT regulates downstream targets through phosphorylation, among which NF-κB, as a downstream target of AKT, is involved in angiogenesis [1]. Currently, there are few studies on PI3K/AKT/NF-κB as targets for angiogenesis therapy.

Tartary buckwheat (Fagopyrum tataricum Gaertn.) has high nutritional value due to its richness in protein, vitamins, dietary fiber, flavonoids, and trace mineral elements [1]. Our previous study successfully isolated and identified three novel antioxidant peptides (Gly-Glu-Val-Pro-Trp, GEVPW; Tyr-Met-Glu-Asn-Phe, YMENF; Ala-Phe-Tyr-Arg-Trp, AFYRW) by alkaline protease from Tartary buckwheat albumin; Among these, AFYRW
exhibited stability and was non-toxic []. Our further research demonstrated that AFYRW hampered the progress of atherosclerosis by reducing the aortic plaque area (data not published). Notably, one therapeutic target for stabilizing atherosclerotic plaques is inhibition of angiogenesis []. However, the relevance of AFYRW and angiogenesis remains unknown.

In the present study, we evaluated the effects of AFYRW on H$_2$O$_2$-induced vascular injury and explored the underlying mechanism, aiming to identify new potential therapeutic targets that augment the effectiveness of treatments. We found that AFYRW alleviates H$_2$O$_2$-induced vascular injury via the PI3K/AKT/NF-κB pathway, demonstrating that AFYRW may serve as a therapeutic option in angiogenesis and providing a basis for the further utilization of Tartary buckwheat.

**Material And Methods**

**Materials**

Tartary buckwheat powder was sold from the Guizhou Weining Tartary Buckwheat Products Co., Ltd. (Guizhou, China). Our previous study isolated antioxidant peptides from Tartary buckwheat albumin, whose sequence was identified by Q-TOF mass spectrometer coupled with an electrospray ionization source as Ala-Phe-Tyr-Arg-Trp (AFYRW) with a molecular mass of 741.85Da (Fig. 1). Subsequently, AFYRW was synthesized by Shanghai Tupper Biotechnology Co., Ltd. (Shanghai, China), and the purity of AFYRW was 95%.

**Cell Lines**

Human umbilical vein endothelial cells (HUVECs) and human acute monocyctic leukemia cells (THP-1) were purchased from ScienCell Research Laboratories (U.S.A). THP-1 cells were maintained in RPMI 1640 medium and HUVECs were maintained in Endothelial Cell Medium supplemented with 10% fetal bovine serum, 1% endothelial growth factors, 100 U/mL penicillin, and 100 U/mL streptomycin (ScienCell, U.S.A) in a 37°C incubator under 5% CO$_2$.

There were one control group and three experimental groups. The experimental groups included the H$_2$O$_2$ group (100 μM H$_2$O$_2$, 2 h; Sigma, U.S.A), the low-dose group (10 μg/mL AFYRW, 24 h + 100 μM H$_2$O$_2$, 2 h), and the High-dose group (60 μg/mL AFYRW, 24 h + 100 μM H$_2$O$_2$, 2 h). After being pretreated with AFYRW for 24 h, HUVECs of experimental groups were treated with H$_2$O$_2$ for 2 h to establish the vascular injury model.

**Cell Viability Assay**

Plated 4x10$^3$ cells per well in a 96-well plate (LABSELECT, China), and performed the assay four times for each group. After treatment with H$_2$O$_2$ and AFYRW, 10% volume of CCK8 (Dalian Meilun Biotechnology
Co., Ltd., China) was added to each well and incubated for 0.5-1 h at 37˚C in a culture incubator. The OD values were measured at 450 nm wavelength.

**Plate Colony Assay**

HUVECs in the logarithmic growth phase were planted in the 6-well plate (LABSELECT, China), and then cultured for 5 days. After treatment with H₂O₂ and AFYRW, HUVECs were fixed in 4% paraformaldehyde (Leagene Biotechnology, China) for 20 min at room temperature. Then, stained with 0.1% crystal (Solarbio, China) violet solution for 1 h. Finally, the number of clones was counted after washing and drying.

**Ros Assay**

HUVECs were cultured in serum-free medium containing 10 μM DCFH-DA (Dalian Meilun Biotechnology Co., Ltd., China) at 37°C in a 5% CO₂ cell incubator for 1 h. After washing, 200 μL PBS was added, and the fluorescence intensity was observed by an inverted fluorescence microscope (Nikon, Japan) and the images were collected.

**Sa-β-gal Staining**

Degree of HUVECs senescence was determined using SA-β-gal staining kits (Solarbio, China), according to the manufacture's protocols. HUVECs were fixed with β-Gal fixative for 15 min at room temperature, and then incubated with staining solution at 37°C overnight. In senescent cells, the SA-β-gal activity was shown by blue staining. The number of senescent cells was observed by a microscope.

**No And Mda Testing**

After treatment with AFYRW and H₂O₂, HUVECs were washed twice with PBS buffer and digested with trypsin. Then, the cells were collected and centrifuged at 1000 rpm for 3 min. Finally, NO and MDA of HUVECs were tested by kits (Nanjing Jiancheng Bioengineering Research Institute, China), according to the manufacture's protocols.

**Matrigel Assay**

Precooled matrigel gel (CORNING, U.S.A) was slowly added to the 96-well plate and incubated at 37°C for 30 min. After digestion, the cell suspension was adjusted to 1×10⁵/mL. 50 μL of the cell suspension was processed and cultured at 37°C in a 5% CO₂ cell incubator for 3 h. The formation of lumen-like structures was observed by an inverted microscope (Nikon, Japan).
Real-time Pcr

Total RNA was extracted and quantified by Nanodrop 2000 (Implen, German). 1 µg of RNA was reverse transcribed to cDNA. Transcripts were quantified by real-time PCR using CFX96 Real-Time Detection System (Applied Biosystems, U.S.A). Gene-specific primers were shown as follows.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5´-3´)</th>
<th>Reverse Primer (5´-3´)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>CCTGGCACCCAGCACAAT</td>
<td>GGGCCGGAACCTCGTCATAAC</td>
</tr>
<tr>
<td>PI3K</td>
<td>TGTGACTTACCAAGAATGGCT</td>
<td>ACCCAGCTACAGGATAATG</td>
</tr>
<tr>
<td>VEGF</td>
<td>ATGGCAGTTGGAGGAAG</td>
<td>CGCTGGATAACAAGGGT</td>
</tr>
</tbody>
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Western Blotting

Protein was extracted and quantified by the BCA kit (Beyotime, China). Equal amounts of protein were separated by SDS-PAGE, transferred onto PVDF membranes (Millipore, U.S.A), and then blocked in the buffer. Primary antibodies (p65 and p-p65: Cell Signaling Technology, U.S.A; PI3K, Akt, VCAM-1, IL-6, and TNF-α: Proteintech, China; VEGF: Abclonal, China) were diluted in blocking buffer. Secondary antibodies were mouse-HRP or rabbit-HRP (Proteintech, China). Blots were observed using Western ECL Blotting Substrate (Dalian Meilun Biotechnology Co., Ltd., China) and imaged. After collecting images, the gray value of each band was analyzed by Image J software.

Scratch Assay

After drawing vertical lines with the same breadth and narrowness in each well, the cells were rinsed 3 times with PBS to get rid of the floating cells. The medium was replaced with serum-free medium and pretreated with AFYRW at 37°C in a 5% CO₂ cell incubator for 24 h, and then photographed under the microscope. Successively, cells were incubated with H₂O₂ for 12 h, and the images were acquired by an inverted microscope at the same position. The changes of scratch healing distance were analyzed by Image J software.

Mononuclear/endothelial Cell Binding

THP-1 cells were centrifuged at 1000 rpm for 3 min, resuspended in RPMI 1640 medium and labeled with BCECF-AM (5 µmol/L). After 30 min, the cells were washed 3 times with PBS to remove excess BCECF-AM (Dalian Meilun Biotechnology Co., Ltd., China), and then THP-1 were co-cultured with HUVECs for 1 h. The non-adherent cells were washed with PBS, and adherent cells were photographed by an inverted fluorescence microscope.
Immunofluorescence

Cells were fixed with 4% paraformaldehyde on ice for 20 min. 5% bovine serum albumin and 0.2% Triton X-100 (Solarbio, China) were used to block nonspecific staining at 4°C for 1 h. Cells were incubated sequentially with primary antibody and fluorescence-labeled secondary antibody (Proteintech, China). Finally, cells were stained with 10 µg/ml of DAPI (Solarbio, China) for 10 min. The images were obtained by laser scanning confocal microscope (Olympus, Japan).

Data analysis

All experiments were repeated at least 3 times. The experimental data of each group was shown as mean ± standard and analyzed by SPSS software. The homogeneity of variance test and OneWay ANOVA were used to compare multiple groups. *P*< 0.05 was considered statistically significant.

Results

AFYRW increased H$_2$O$_2$-induced cell viability

To determine whether AFYRW is involved in regulating cell viability of H$_2$O$_2$-induced HUVECs, we used the CCK8 assay. The results showed that 5-100 µg/mL AFYRW for 24 h had no impact on cell viability, indicating that AFYRW is not cytotoxic (Fig. 2A). In addition, cell viability was unaffected by 50µmol/L H$_2$O$_2$, but was substantially reduced by 100–200 µmol/L H$_2$O$_2$ for 2 h (Fig. 2B). We next pretreated HUVECs with 5-100 µg/mL AFYRW for 24 h and then stimulated HUVECs with 100 µmol/L H$_2$O$_2$ for 2 h. As expected, AFYRW, also significantly improved the cell viability during induction with H$_2$O$_2$ (Fig. 2C). So, we selected 100 µmol/L H$_2$O$_2$ for 2 h, 10 µg/mL and 60 µg/mL AFYRW for 24 h in subsequent experiments.

Plate colony assay was further used to validate the effects of AFYRW on H$_2$O$_2$-induced cell viability. The number of cell colonies in the H$_2$O$_2$ treated group (100 µmol/L, 2 h) was significantly lower than that in the control group, while 10 µg/mL or 60 µg/mL AFYRW for 24 h improved the number of colonies (Fig. 2D-E), which was consistent with the CCK8 assay. These results demonstrate that AFYRW significantly increases H$_2$O$_2$-induced cell viability.

Afyrw Decreased The Expression Of Pi3k And The Phosphorylation Of Akt

Activated PI3K stimulates AKT, which in turn participates in angiogenesis through phosphorylation to regulate downstream targets []. To identify the role of AFYRW on the expression of PI3K and p-AKT in HUVECs, we performed real-time PCR and western blotting to measure their levels. As shown in Fig. 3, AFYRW (10 or 60 µg/mL, 24 h) significantly prevented the H$_2$O$_2$ (100 µmol/L, 2 h) mediated upgrade of
PI3K and p-AKT. These findings suggest that AFYRW is an inhibitor of H$_2$O$_2$-induced PI3K expression and AKT phosphorylation.

**Effects Of Afyrw On The Ros Level, Oxidative Stress, And Aging In H$_2$O$_2$-treated Huvecs**

In addition to being an important regulator of angiogenesis, ROS also plays a role in the control of the PI3K/Akt cell signaling pathway [1]. Therefore, we sought to determine the effects of AFYRW on ROS levels and cell senescence. AFYRW (10 or 60 µg/mL, 24 h) significantly hampered the H$_2$O$_2$-induced (100 µmol/L, 2 h) up-regulation of ROS levels in HUVECs (Fig. 4A-B). Next, we utilized β-galactosidase (SA-β-gal) staining to detect the degree of cellular senescence. The result showed that AFYRW seriously inhibited H$_2$O$_2$-induced cell senescence (Fig. 4C-D).

ROS promotes angiogenesis by generating reactive oxidation products. NO and MDA can be used to measure cellular lipid peroxidation. We doubted whether NO and MDA might be subjected to AFYRW regulation, so we detected their expression levels in H$_2$O$_2$-induced HUVECs. The results demonstrated that after H$_2$O$_2$ treatment (100 µmol/L, 2 h), the levels of NO and MDA in the supernatant of cell culture medium were greatly increased, while AFYRW (10 or 60 µg/mL, 24 h) seriously down-regulated H$_2$O$_2$-induced NO and MDA expression (Fig. 4E-F). Our finding provided evidence that AFYRW could reduce ROS level, improve cellular oxidative stress state, and delay endothelial cell senescence.

**Afyrw Restrained Vegf Expression And Angiogenesis**

PI3K, as an important regulator of angiogenesis, is involved in endothelial cell tube formation in vitro [4]. VEGF promotes vascular endothelial cell proliferation and capillary permeability, and activates PI3K through a variety of pathways [5]. So, the effects of AFYRW for VEGF expression and angiogenesis were assessed. The formation of tube-like structures was markedly increased in H2O2-induced (100 µmol/L, 2 h) HUVECs, while AFYRW (10 or 60 µg/mL, 24 h) decreased H$_2$O$_2$-induced angiogenesis in HUVECs (Fig. 5A-B). Consistent with our observation of angiogenesis, AFYRW hampered H$_2$O$_2$-induced VEGF expression in HUVECs (Fig. 5C-E). Overall, AFYRW restrained VEGF expression and angiogenesis.

**Afyrw Hampered The Cell Migration And Invasion In Huvecs**

The key processes in the initiation of angiogenesis are the proliferation, migration, differentiation, and tube formation of vascular endothelial cells [4]. To determine whether AFYRW was involved in the regulation of cell migration and invasion in HUVECs, we examined the effect of AFYRW on cell migration and invasion. This result suggested that H$_2$O$_2$ (100 µmol/L, 2 h) significantly enhanced cell migration, while H$_2$O$_2$ plus AFYRW (10 or 60 µg/mL, 24 h) suppressed cell migration greatly compared with H$_2$O$_2$ alone (Fig. 6A-B). In another similarly designed experiment, we found that AFYRW suppressed the cell
invasion in H_2O_2-induced HUVECs (Fig. 6C-D). In summary, AFYRW hampered the migration and invasion of HUVECs.

**Impacts Of Afyrw On The Nuclear Translocation Of Nf-κb, Adhesion Molecules And Inflammatory Factors Expression**

NF-κB, as a downstream of PI3K/Akt, plays a key role in inflammatory responses. After NF-κB is activated, p65 translocation enters the nucleus and participates in the transcription of target genes []. However, the relevance between AFYRW and NF-κB expression in H_2O_2-induced HUVECs remains unknown. To test their association, we utilized western blotting and immunofluorescence to measure the expression of p65 and NF-κB respectively. Compared with controls, H_2O_2 (100 µmol/L, 2 h) promoted p65 phosphorylation, which was significantly inhibited by AFYRW (10 or 60 µg/mL, 24 h; Fig. 7A-B). The results of immunofluorescence showed that H_2O_2 also promoted the nuclear translocation of NF-κB, while AFYRW restored that (Fig. 7C).

After activation of NF-κB, a variety of adhesion molecules and pro-inflammatory cytokines are secreted, which attract monocytes to adhere to the vascular endothelium. Next, we examined the related inflammatory factors expression and cell adhesion ability in HUVECs. The expression levels of TNF-α, IL-6 and VCAM-1 were significantly increased after H_2O_2 (100 µmol/L, 2 h) treatment, while AFYRW (10 or 60 µg/mL, 24 h) decreased the H_2O_2-induced up-regulation of inflammatory factors (Fig. 8A-B). Similarly, AFYRW restrained monocyte-endothelial adhesion induced by H_2O_2 (Fig. 8C-D). Our finding provided evidence that AFYRW improved H_2O_2-induced inflammatory responses by inhibiting NF-κB signaling.

**Discussion**

Angiogenesis originates from the existing microvascular endothelium, which is the main way of neovascularization in adults []. Most cells involved in the formation of blood vessels, such as endothelial cells and smooth muscle cells, produce ROS. ROS, as an important regulator in angiogenesis, stimulates the angiogenic response during ischemia-reperfusion of the heart []. Furthermore, Rosmarinic acid inhibits angiogenesis by lowering intracellular ROS levels, H2O2-dependent VEGF expression, and IL-8 levels []. Certain inflammatory mediators, such as NO, promote the synthesis of VEGF []. In the present study, AFYRW significantly inhibited the H2O2-induced up-regulation of ROS levels, cellular senescence, and contents of NO and MDA in HUVECs, suggesting AFYRW may be a suppressor for vascular injury.

Activated PI3K promotes cell proliferation and invasion by facilitating Akt phosphorylation []. The PI3K/Akt pathway is regulated by ROS, which is closely related to tumor angiogenesis, invasion, and metastasis [6]. Apigenin hampers tumor angiogenesis by inhibiting the expression of HIF-1a and VEGF in cells through the PI3K/Akt pathway []. Here, we have identified that AFYRW significantly suppressed the H2O2-induced rise of PI3K and p-Akt levels in HUVECs, indicating AFYRW regulates angiogenesis possibly through the PI3K/Akt pathway.
As a nuclear transcription factor, NF-κB plays vital roles in adjusting the expression of inflammatory factors, growth factors, and adhesion molecules [1]. Notably, NF-κB is involved in angiogenesis as a downstream target of Akt. Our analysis revealed that H2O2 stimulated nuclear translocation of NF-κB and p65 phosphorylation in HUVECs, which was significantly inhibited by AFYRW. Moreover, AFYRW largely down-regulated the H2O2-induced increase of VCAM-1, TNF-α, and IL-6 levels in HUVECs. Overall, AFYRW reduced the expression levels of adhesion molecules and inflammatory factors by inhibiting the PI3K/AKT/NF-κB signaling, thus slowing the inflammatory response.

Tumor growth and metastasis depend on angiogenesis [1]. Our results suggested that H2O2 markedly boosted tube-like structures, cell migration, and migration, while AFYRW suppressed that in H2O2-induced HUVECs. VEGF greatly promotes the proliferation of vascular endothelial cells, suggesting that VEGE plays an active role in oncogenesis [25]. We next examined whether VEGF expression might be subjected to AFYRW regulation in H2O2-induced HUVECs, and our results demonstrated that VEGF expression and angiogenesis were significantly inhibited after AFYRW treatment.

**Conclusion**

Together our data point toward the conclusion that H2O2-induced angiogenesis impairment and the inflammatory response are ameliorated by AFYRW through the PI3K/AKT/NF-κB pathway, and a proposed molecular mechanism is shown in Fig. 9. A deep insight into the protective mechanism of AFYRW in angiogenesis may provide data support for the further development and utilization of Tartary buckwheat protein-derived peptide, and thus offer new therapeutic options for vascular injury.

**Abbreviations**

AKT: protein kinase B; H2O2: Hydrogen peroxide; HUVECs: Human umbilical vein endothelial cells; IF: Immunofluorescence; IL-6: Interleukin 6; MDA: Malondialdehyde; NF-κB: nuclear factor-κB; NO: Nitric oxide; PI3K: phosphoinositide 3-kinase; ROS: reactive oxygen; TNF-α: Tumor necrosis factor α; VCAM-1: Vascular cell adhesion molecule 1; VEGF: Vascular endothelial growth factor; β-gal: senescence-associated β galactosidase.

**Declarations**

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**Author Contributions**
Yi Xiao: Writing-original draft, Methodology, Investigation, Data analysis. Jiajun Yang: Funding acquisition, Writing–original draft, review and editing, Investigation; Yan Deng: Methodology, Investigation, Data analysis; Lilin Zhang: Investigation, Data analysis; Qingzhong Xu: Data analysis; Hongmei Li: Funding acquisition, Writing-review and editing, Methodology, Investigation, Supervision.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

References


**Figures**

![Figure 1](image1.png)

**Figure 1**

Figure 2

Effects of AFYRW on cell viability in H\(_2\)O\(_2\)-induced HUVECs. A, Effect of AFYRW (5-100 μg/mL, 24 h) on cell viability in HUVECs. B, Effect of H\(_2\)O\(_2\) (50-200 μmol/L, 2 h) on cell viability in HUVECs. C, AFYRW pretreatment (5-100 μg/mL, 24 h) improved cell viability in H\(_2\)O\(_2\)-induced (100 μmol/L, 2 h) HUVECs. D-E, Plate colony of AFYRW (10 or 60 μg/mL, 24 h) on H\(_2\)O\(_2\)-induced (100 μmol/L, 2 h) HUVECs (C) and the number of cell colonies (C).
Figure 3

The expression levels of PI3K and p-AKT. A-B, Western blotting of PI3K protein levels in AFYRW and H₂O₂-treated HUVECs. C-D, Western blotting of p-AKT protein levels in AFYRW and H₂O₂-treated HUVECs. E, PI3K mRNA levels in AFYRW and H₂O₂-treated HUVECs were assessed by real-time PCR.
Figure 4

The antioxidant capacity of AFYRW. A-B, Effect of AFYRW on H$_2$O$_2$-induced ROS in HUVECs. C-D, Staining of senescent cells in AFYRW and H$_2$O$_2$-treated HUVECs. E-F, Effects of AFYRW on the content of NO (E) and MDA (F).
Figure 5

Effects of AFYRW on angiogenesis. A-B, Effect of AFYRW on angiogenesis in \( \text{H}_2\text{O}_2 \)-treated HUVECs. C-D, Effect of AFYRW on VEGF protein levels in \( \text{H}_2\text{O}_2 \)-treated HUVECs. E, Effect of AFYRW on VEGF mRNA levels in \( \text{H}_2\text{O}_2 \)-treated HUVECs.
Figure 6

A-D, Effects of AFYRW on cell migration (A-B) and invasion (C-D) in H₂O₂-induced HUVECs.
Figure 7

Effects of AFYRW on NF-κB. A-B, effect of AFYRW on p65 expression levels in H$_2$O$_2$-induced HUVECs. C, effect of AFYRW on nuclear translocation of NF-κB in H$_2$O$_2$-induced HUVECs.
Figure 8

Detection of inflammatory factors and adhesion ability. A-B, Western blotting of VCAM-1, TNF-α, and IL-6 protein levels in AFYRW and H$_2$O$_2$-treated HUVECs. C-D, Effects of AFYRW on monocyte-endothelial adhesion in H$_2$O$_2$-induced HUVECs.
Figure 9

The proposed molecular mechanism.