Biomedical Research and Therapy 2024, 11(4):6339-6347

Open Access Full Text Article

Original Research

Kaempferol Rescues Vascular Endothelial Ferroptosis by Inhibiting Lipid Peroxidation

Li Wen[1,#], Wei-yuan Zhang[2,3,#], Li-sheng Wang[1,2,*], Feng-jun Xiao[4,*]

ABSTRACT

Introduction: The vascular endothelium plays a pivotal role in maintaining vascular function and physiological balance. The degradation and injury of endothelial cells are critical pathological events in the progression of vascular diseases, leading to cell death. One such cell death mechanism, ferroptosis, is an iron-dependent form of necrosis characterized by extensive lipid peroxidation-mediated membrane damage and the toxic effects of iron and lipid peroxidation. Kaempferol, a flavonoid, is celebrated for its antioxidant, anti-inflammatory, and anti-cancer properties. Despite these benefits, the impact of Kaempferol on endothelial cell ferroptosis and its potential therapeutic applications in vascular diseases have yet to be fully elucidated. **Methods**: Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay. Oxidative stress and lipid peroxidation were measured using Dihydroethidium (DHE) and C11-BODIPY 581/591, respectively. The protein and RNA levels of ferroptosis-associated molecules, including solute carrier family 7 member 11 (SLC7A11) and glutathione peroxidase 4 (GPX4), were determined through Western blotting and real-time fluorescence quantitative polymerase chain reaction (qPCR). **Results**: Treatment with a glutathione peroxidase 4 inhibitor (RSL3) led to rapid cytotoxicity in human umbilical vein endothelial cells (HUVECs). Notably, Kaempferol demonstrated a significant protective effect against RSL3-induced ferroptosis in HUVECs. Kaempferol treatment reduced the accumulation of reactive oxygen species (ROS) and exhibited distinctive morphological changes associated with ferroptosis. Moreover, Kaempferol treatment resulted in the upregulation of SLC7A11 and GPX4 expression in HUVECs, highlighting its potent ability to mitigate ferroptosis among tested flavonoids. **Conclusions**: Kaempferol effectively inhibited RSL3-induced ferroptosis in HUVECs by modulating the expression of SLC7A11 and GPX4, thereby reducing lipid peroxidation. These findings underscore the therapeutic potential of Kaempferol in the treatment of vascular diseases, paving the way for its application in clinical settings.

Key words: kaempferol, lipid oxidation, ferroptosis, SLC7A11, GPX4

# These authors contribute equally to the work

[1]*School of Nursing, Jilin University, Changchun, 130021, PR China*

[2]*Laboratory of Molecular Diagnosis and Regenerative Medicine, the Affiliated Hospital of Qingdao University, Qingdao, 266000, PR China*

[3]*Department of Special Medicine, School of Basic Medicine, Qingdao University, Qingdao, 266071, PR China*

[4]*Beijing Institute of Radiation Medicine, Beijing, 100850, PR China*

Correspondence

Li-sheng Wang, School of Nursing, Jilin University, Changchun, 130021, PR China

Laboratory of Molecular Diagnosis and Regenerative Medicine, the Affiliated Hospital of Qingdao University, Qingdao, 266000, PR China

Email: lishengwang@jlu.edu.cn

Correspondence

Feng-jun Xiao, Beijing Institute of Radiation Medicine, Beijing, 100850, PR China

Email: xiaofjun@sina.com

INTRODUCTION

The vascular endothelium, which is dynamic, diverse, and widespread, plays a crucial role in secretion, production, breakdown, and defense mechanisms[1,2]. Endothelial cells (ECs), forming the innermost layer of all blood vessels, have direct exposure to chemicals or particles within the circulatory system. They are pivotal in promoting multi-organ health and homeostasis through the regulation of solute permeability, shear stress response, vasodilatory tone maintenance, and their ability to exhibit both anti-inflammatory and pro-inflammatory, as well as antioxidant and pro-oxidant activities[3,4]. Evidence increasingly supports the involvement of endothelial cell death in the onset and progression of vascular diseases[5–7].

Cell death, an evolutionary conserved process, serves to regulate cell populations by eliminating excessive, damaged, or senescent cells[8]. Among the mechanisms of cell death, regulated cell death (RCD) stands out as crucial for maintaining tissue equilibrium and is implicated in a multitude of diseases. RCD includes both apoptotic and non-apoptotic forms[9], with several non-apoptotic RCDs identified, such as necroptosis, ferroptosis, pyroptosis, and autophagy-dependent cell death[10]. Ferroptosis, a type of iron-dependent regulated necrosis, stems from extensive lipid peroxidation-induced membrane damage, causing iron and lipid peroxidation toxicity. This process is evolutionarily conserved and vital in both the development and pathogenesis of diverse organisms, including plants and animals[11]. Ferroptosis regulation involves enzymes like acyl-CoA synthetase long-chain family member 4 (ACSL4), lysophosphatidylcholine acyltransferase 3 (LPCAT3), arachidonic acid lipoxygenases (ALOXs), and glutathione peroxidase 4 (GPX4)[12], highlighting the potential of targeting endothelial cell death in vascular disease treatments.

Chinese medicines and their active components offer a novel approach to modulating ferroptosis, char-

Cite this article : Wen L, Zhang W, Wang L, Xiao F. **Kaempferol Rescues Vascular Endothelial Ferrop-tosis by Inhibiting Lipid Peroxidation**. *Biomed. Res. Ther.* 2024; 11(4):6339-6347.

History
- Received: Jan 26, 2024
- Accepted: Mar 20, 2024
- Published Online: Apr 30, 2024

DOI : 10.15419/bmrat.v11i4.880

acterized by diverse regulatory targets, structural stability, high safety profile, and affordability. Various traditional Chinese medicine ingredients have shown efficacy in disease treatment by targeting ferroptosis pathways. For example, luteolin inhibits ferroptosis in cardiac microvascular endothelial cells by enhancing interferon regulatory factor (IRF) in the context of cardiac hypertrophy [13]. Similarly, procyanidins (PCs) counteract oxidative stress and ferroptosis through the activation of the nuclear factor erythroid-derived 2-like 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway [14]. Investigating Chinese medicines' intervention mechanisms in ferroptosis opens new avenues for the research and development of innovative disease therapies [15].

Flavonoids, recognized for their potent antioxidant, anti-inflammatory, anti-cancer, and anti-viral properties [16], are abundantly found in fruits, vegetables, and tea. Their medicinal benefits make them integral to pharmaceuticals, dietary supplements, and beauty products [17]. Liu L *et al.* have comprehensively reviewed the regulatory functions of natural flavonoids on ferroptosis, underscoring their clinical therapy potential [18]. Kaempferol, abundant in plant-based foods like kale, broccoli, beans, spinach, and tea [19], illustrates the therapeutic spectrum of flavonoids, including anti-oxidative [16], anti-inflammatory [20], and anti-cancer effects [21]. Its efficacy in managing conditions such as diabetes mellitus [22], atherosclerosis [23], and osteoporosis [24] has been well-documented. Furthermore, kaempferol's neuroprotective [25] and liver [26] and myocardium [27] benefits position it as a promising candidate for alleviating inflammatory responses [28]. Despite these findings, the specific impact of kaempferol on endothelial cell ferroptosis and its potential in vascular disease therapy warrants further exploration. This study aims to elucidate kaempferol's protective mechanisms against endothelial cell ferroptosis.

METHODS

Cell Culture

In our study, we utilized Human Umbilical Vein Endothelial Cells (HUVECs) sourced from our research group's cell bank. The culture method we employed was based on the protocol described by Li *et al.* [29]. We maintained HUVECs in a controlled environment at 37°C within a 5% CO_2 incubator, using Dulbecco's Modified Eagle's Medium (DMEM; C11885500BT, Gibco), enriched with 10% Fetal Bovine Serum (FBS; FSP500, ExCell) and 1% Penicillin-Streptomycin Solution (P1400, Solarbio).

Cytotoxicity Assessment

For assessing cytotoxicity, we seeded HUVECs in 96-well plates and treated them with varying concentrations of kaempferol (ranging from 0.625 to 40 μM in DMSO) for 24 hours. To evaluate cell viability, we added 10 μl of the Cell Counting Kit-8 (CCK-8; 40203ES60, YEASEN) solution to each well and incubated them for 3 hours at 37°C in a 5% CO_2 environment. Subsequently, we measured the absorbance at 450 nm using a spectrophotometer.

Cell Survival Experiment

For the cell survival experiment, HUVECs were plated in 96-well plates and treated either with the GSH peroxidase 4 inhibitor RSL3 (Y-100218A, MCE) or kaempferol. After incubation at 37°C in a 5% CO_2 incubator for 3 hours, we measured absorbance at 450 nm using a spectrophotometer for cell viability assessment.

Detection of Reactive Oxygen Species (ROS)

To detect ROS production, we utilized the Superoxide Anion Probe Dihydroethidium (DHE) assay [30]. This involved culturing HUVECs and subsequently incubating them with 10 μM DHE (S0063, Beyotime) at 37°C for 30 minutes. After washing the cells twice with phosphate-buffered saline (PBS) and fixing them with 4% paraformaldehyde for 30 minutes, we applied an anti-fluorescence quenching agent containing 4',6-diamidino-2-phenylindole (DAPI) (ZLI-9556, ZSGB-BIO) for counter-staining. We then examined and photographed the cells under a confocal microscope.

Lipid Peroxidation Measurement

Following the methodology of Mei *et al.* [31], we detected lipid peroxidation using the C11 BODIPY 581/591 indicator. After pretreating HUVECs, we added C11 BODIPY 581/591 (D3861, Invitrogen) at a final concentration of 5 μM to the culture medium and co-incubated it for one hour at 37°C. We washed the cells twice with PBS, treated them with trypsin, resuspended them in PBS containing 5% FBS, and finally analyzed them using flow cytometry.

Western Blot Analysis

We analyzed the intracellular protein content using the Western Blot technique [32]. After pretreatment, HUVECs were lysed with RIPA buffer containing protease inhibitors on ice for 30 minutes. The proteins were then separated by SDS-PAGE and

transferred onto PVDF membranes. After blocking with 5% skim milk, the membranes were incubated with primary antibodies against SLC7A11 (ab175186, Abcam), GPX4 (A1933, ABclonal), and GAPDH (A19056, ABclonal) overnight at 4°C, using a 1:1000 dilution. The next day, we incubated the membranes with an HRP-conjugated Goat Anti-Rabbit secondary antibody (RGAR001, Proteintech) at a 1:10,000 dilution for one hour at room temperature. Detection was achieved using a chemiluminescent substrate. GAPDH served as a loading control[33,34], and the bands were quantitatively analyzed using ImageJ software (version 1.4.3.67).

Realtime Fluorescence Quantitative PCR (qPCR)

We extracted total RNA using TRI Reagent (T9424, Sigma) and synthesized cDNA with TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix (AT311-02, Transgen). We performed qPCR using the QuantStudioTM 3 System and PerfectStart® Green qPCR SuperMix (+Universal Passive Reference Dye) (AQ602-01, Transgen), detecting fluorescence with SYBR Green. The amplification efficiency was calculated as $E = 10^{(-1/k)}$-1, with efficiencies for SLC7A11 and GPX4 at 99.8% and 102.7%, respectively. We normalized the expression levels of SLC7A11 and GPX4 mRNAs to β-actin mRNA using the $2^{-\Delta\Delta Ct}$ method and employed the following primers for qPCR:

- **β-actin:**
- Forward: 5′-CCTGGCACCCAGCACAAT-3′
- Reverse: 5′-GGGCCGGACTCGTCATAC-3′
- **SLC7A11:**
- Forward: 5′-ATGCAGTGGCAGTGACCTTT-3′
- Reverse: 5′-CATGGAGCCAAAGCAGGAGA-3′
- **GPX4:**
- Forward: 5′-GAAGATCCAACCCAAGGGCA-3′
- Reverse: 5′-GACGGTGTCCAAACTTGGTG-3′

Statistical Analysis

We meticulously analyzed all data to ensure a normal distribution and presented the results as the mean ± standard deviation (SD). Statistical significance was determined using Student's t-test or one-way ANOVA, followed by post-hoc testing. We utilized Pearson's product-moment correlation for correlation analyses. All statistical procedures were conducted using GraphPad Prism version 8.0.2, considering p-values of < 0.05 as statistically significant.

RESULTS

Kaempferol Protects HUVECs from RSL-3-Induced Ferroptosis

Ferroptosis was induced in HUVECs using different concentrations of RSL-3 (DMSO, 0.125, 0.25, 0.5, and 1 μM). A dose-dependent decrease in cell viability in HUVECs was detected. The LD50 of RSL-3 in cell viability was achieved at a concentration of 0.25 μM. Subsequent induction experiments were carried out using this concentration (**Figure 1**A). To screen for the most active flavonoids, HUVECs were treated with different flavonoid components and co-cultured with RSL-3 for 24 hours. Kaempferol demonstrated the strongest ability to rescue the RSL-3-induced ferroptosis (**Figure 1**B). **Figure 1**C shows the chemical structural formula of Kaempferol.

Kaempferol Shows Low Toxicity in HUVEC Culture

The toxicity of kaempferol on HUVECs was analyzed using the CCK-8 assay. HUVECs were treated with different concentrations of kaempferol (DMSO, 0.625, 1.25, 2.5, 5, 10, 20, and 40 μM) for 24 hours, and the cell viability was analyzed. Interestingly, kaempferol showed no toxicity, but a slight growth-promoting effect (**Figure 2**A). Moreover, kaempferol was shown to resist ferroptosis and effectively rescue HUVECs similar to the control group at a concentration of 5 μM. The subsequent experiments were performed with this concentration to rescue HUVEC ferroptosis (**Figure 2**B).

Kaempferol Reduces ROS Generation in RSL-3-Treated HUVECs

Ferroptosis is a ROS-dependent, non-apoptotic, lipid-peroxidation-induced cell death closely related to the intracellular ROS content[35,36]. ROS are generated during normal physiological processes and are essential for cell signaling and tissue homeostasis[37]. The C11 BODIPY 581/591 and DHE assays were used to determine the lipid oxidation profiles and oxidative stress in HUVECs, respectively. The results showed that the fluorescence intensity of DHE increased significantly following RSL-3 treatment and C11 BODIPY 581/591 addition, whereas both DHE and C11 BODIPY 581/591 decreased significantly upon treatment with kaempferol, indicating the antioxidant capacity of kaempferol (**Figure 3**).

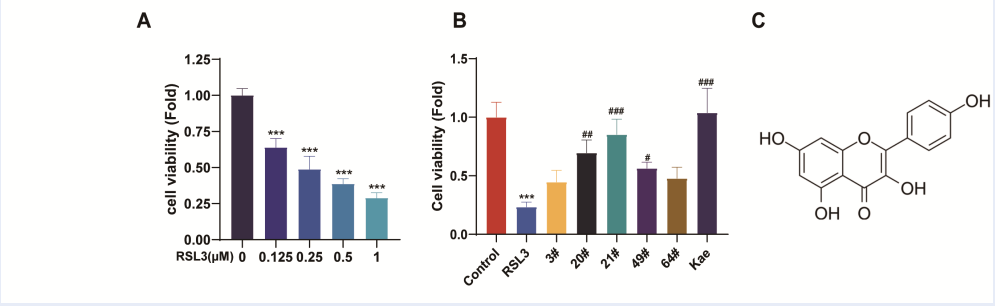

Figure 1: **Anti-ferroptosis assay of kaempferol. A**. Cell survival was determined by CCK8 assay after treatment of human umbilical vein endothelial cells (HUVECs) with erent concentrations of GSH peroxidase 4 inhibitor (RSL3) for 24 h (n=5). *vs control. **B**. Cell survival was determined by co-culturing vonoid fractions with RSL3 for 24 h (n=3). *vs control #vs RSL3. C. Structural formula of kaempferol. (***p<0.001, #p<0.05, ##p<0.01, ###p<0.001).

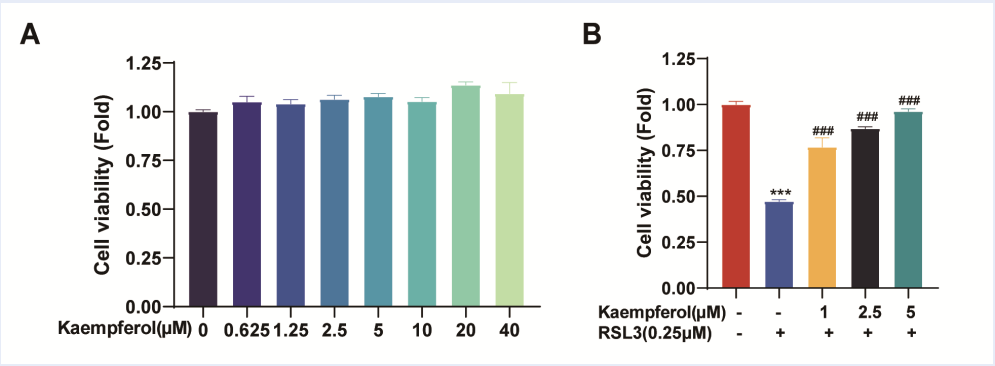

Figure 2: **Kaempferol shows low toxicity**. **A**. Cell survival was determined after 24 h treatment ofHUVECs with erent concentrations of kaempferol to detect the kaempferoltoxicity (n=5). **B**. Cell survival was determined after treating HUVECs with RSL3 (0.25 μM) plusDMSO and kaempferol (1, 2.5, and 5 μM) for 24 h (n=5). *vs control, #vs RSL3 (###p<0.001,***p<0.001).

Kaempferol Inhibits Ferroptosis by Upregulating SLC7A11 and GPX4

The protein expression of SLC7A11 and GPX4 was significantly downregulated in the RSL-3 group. However, treatment with kaempferol restored the protein levels to that of the control, suggesting that kaempferol inhibits ferroptosis in HUVECs (**Figure 4A, B, C**). This correlation was also verified at the RNA level, with qPCR results being consistent with the western blot findings (**Figure 4 D, E**).

DISCUSSION

The pathology of vascular diseases often involves the dysregulation of endothelial cell death, making the study of endothelial cell death models crucial for the identification of effective treatments for such diseases. Human Umbilical Vein Endothelial Cells (HUVECs) are frequently utilized in research on cell biology within the contexts of angiogenesis, vascular diseases, and cardiovascular diseases[38]. Due to their superior proliferation and migration abilities, as well as their capacity to form *in vitro* tubular structures resembling angiogenesis, HUVECs are considered ideal models for exploring endothelial cell death[38].

Ferroptosis, a form of non-apoptotic cell death characterized by iron-dependent lipid peroxidation, is distinguished by an accumulation of lipid peroxides leading to cell swelling and the subsequent rupture of the cell membrane[8,39]. The process of lipid peroxidation is fundamental to ferroptosis[40]. Compounds such as RSL3 and erastin, known inducers of ferroptosis, are employed to develop cell models for this form of cell death. Erastin targets System Xc- activity to disrupt glutathione (GSH) synthesis, a pathway leading to ferroptosis[41], whereas RSL3, by directly inhibiting GPX4, activates iron-dependent, nonapoptotic cell death in cells with RAS mutations[42,43] and in various cell types[44,45]. Observations of morphologi-

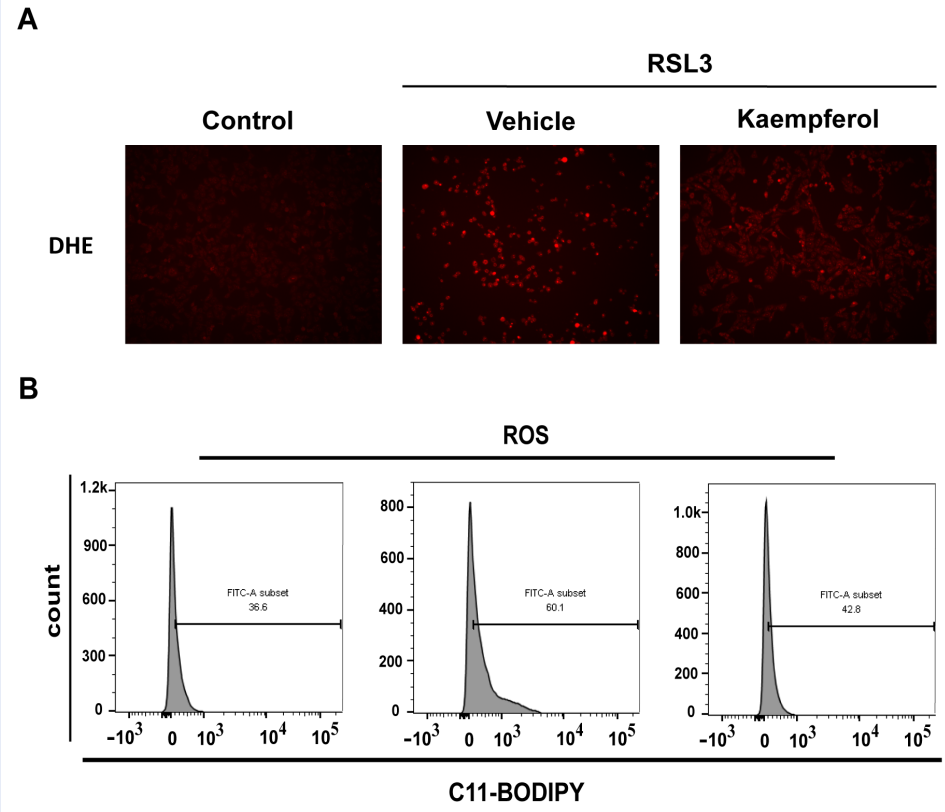

Figure 3: **The role of kaempferol in inhibiting lipid oxidation**. HUVECs were treated with RSL3 (0.25 μM) plus DMSO and kaempferol (5 μM) for 24 h for subsequent assays. The antioxidant capacity of kaempferol was evaluated by DHE (**A**) and C11 BODIPY 581/591 (**B**) assays.

cal changes, including cell and mitochondrial shrinkage as well as cell membrane damage, in HUVECs treated with RSL3 confirm its efficacy in creating an ideal model for endothelial cell ferroptosis.

Recent decades have seen the identification of pharmacological and natural compounds capable of modulating ferroptosis[46,47]. Among these, kaempferol, a compound found in abundance in fruits, vegetables, and herbs, stands out for its minimal toxicity and promising therapeutic potential[48]. Its mechanisms involve the promotion of free radical scavenging, enhancement of antioxidant enzyme activities against lipid peroxidation, and prevention of hemolysis[49]. Moreover, kaempferol acts protectively in ischemic stroke by activating specific signaling pathways (Nrf2/SLC7A11/GPX4) to mitigate oxygen-glucose deprivation/reperfusion-induced cellular damage and suppress ferroptosis initiation[50]. Additionally, it reverses adverse effects such as hepatic iron overload and oxidative stress induced by acetaminophen in mice, showcasing its ability to reduce intracellular ROS accumulation, trigger the Nrf2 pathway, upregulate GPX4, and prevent hepatocyte ferroptosis[51]. Through our research, using HUVECs as a model, we established kaempferol's efficacy in attenuating RSL3-induced cell death, highlighting its potential in the treatment of vascular diseases through ferroptosis inhibition.

In exploring ferroptosis further, we discovered it to be a reactive oxygen species (ROS)-dependent cell demise mechanism, exacerbating oxidative damage through excessive ROS generation via the Fenton reaction[52,53]. Our investigation into the impact of kaempferol on lipid peroxidation, utilizing assays like DHE for intracellular ROS levels[30] and C11-BODIPY for lipid peroxidation[40], confirmed significant inhibition of RSL3-induced lipid peroxidation in HUVECs. Multiple regulatory signals such as GPX4 and SLC7A11 are involved in the regulation of cell ferroptosis[54]. The GSH–GPX4 limits membrane lipid peroxidation via targeting System Xc^- cystine/glutamate antiporter[55,56]. SLC7A11 maintains the production

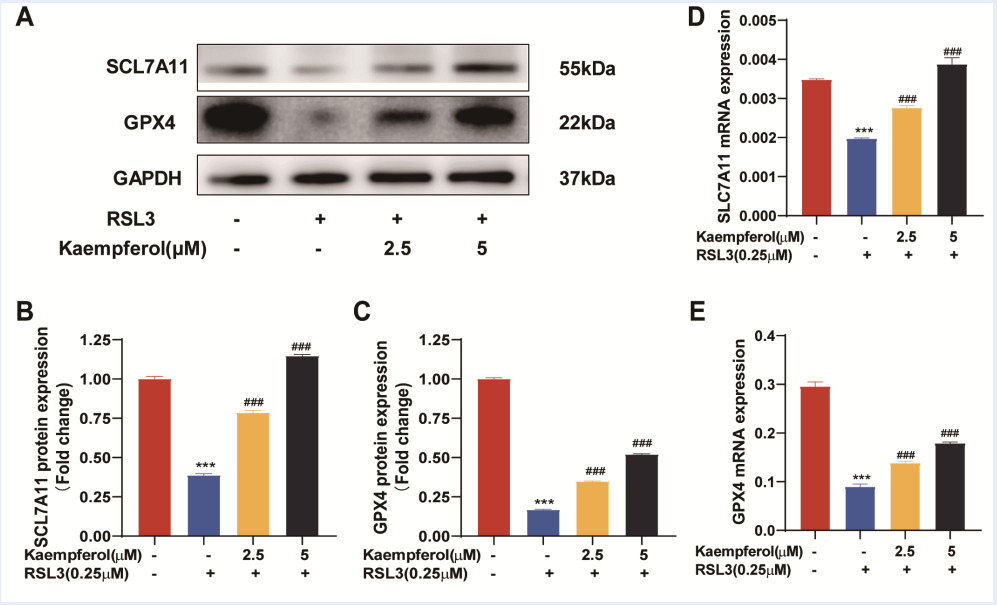

Figure 4: **Kaempferol regulates ferroptosis-associated proteins to inhibit ferroptosis**. HUVECs were treated with RSL3 (0.25 μM) plus DMSO and kaempferol (2.5 μM and 5 μM) for 24 h for subsequent assays. **A**. Protein expression of ferroptosis-associated proteins detected by Western blot analysis. **B**. Gray scale value analysis of solute carrier family 7 member 11 (SLC7A11). **C**. Gray scale value analysis of glutathione peroxidase 4 (GPX4). **D**. Expression of SLC7A11 at the RNA level. **E**. Expression of GPX4 at the RNA level *vs control, $^{\#}$vs RSL3 ($^{\#\#\#}$p<0.001,***p<0.001)

of GSH, a major endogenous antioxidant, through a series of reactions involving the exchange of extracellular cysteine with intracellular glutamate[46]. Inhibiting the SLC7A11 pathway stands out as a critical upstream mechanism for inducing ferroptosis[57]. The expression of GPX4 and SLC7A11 at both protein and RNA levels was investigated in RSL3-treated HUEVCs. This study also provides evidence that kaempferol could significantly protect HUEVCs ferroptosis through the regulation of GPX4 and SLC7A11 expression.

The implications of endothelial cell ferroptosis extend to a variety of vascular-related conditions, including peripheral vascular disease[58], stroke[59], heart disease[60], diabetes[61], venous thrombosis[62], tumor growth[63], and metastasis[64], making the targeting of endothelial cell ferroptosis a novel therapeutic strategy. Kaempferol's multi-faceted pharmacological effects, combined with its minimal toxicity, endow it with significant potential in both health food and pharmaceutical sectors[51].

In previous studies, kaempferol has shown potential effectiveness in the treatment of diseases such as Alzheimer's disease[65] and colon cancer. It exhibits various effects such as antioxidant, anti-inflammatory, anti-tumor, and promotion of glucose metabolism by regulating multiple signaling pathways such as Nrf2/SLC7A11/GPX4, Toll-like receptor 4 (TLR4)/ nuclear factor kappa-B (NF-κB), immunoglobulin-regulated enhancer 1 (IRE1)/ c-Jun N-terminal kinase (JNK)/ C/EBP homology protein (CHOP), and mitogen-activated protein kinases (MAPKs). In addition, compared with some chemotherapeutic agents, kaempferol is not toxic to normal cells[66] and appears to be relatively safe at certain doses[67]. However, clinical trials of kaempferol on humans are still scarce and remain controversial, as most studies are based on animal models or *in vitro* experiments. More studies are still needed to determine its safety, pharmacokinetics and potential adverse effects in humans. In addition, although kaempferol has shown potential therapeutic effects *in vitro* and *in vivo* models, there is a lack of clinical trial validation, and therefore more human studies are needed to confirm its efficacy and safety in clinical applications.

The present study had a limited experimental model and did not elucidate the molecular mechanisms of kaempferol in depth. Future studies could further explore the molecular mechanisms and interactions of kaempferol in regulating signaling pathways and inhibiting iron death, as well as the targets and biolog-

ical effects of kaempferol. This will contribute to a better understanding of the mechanism of action of kaempferol and provide a more scientific basis for its future clinical applications.

CONCLUSIONS

We uncovered the protective role of kaempferol in safeguarding human umbilical vein endothelial cells (HUVECs) from ferroptosis, an iron-dependent form of cell death. This protective mechanism functions through the modulation of GPX4 and SLC7A11, crucial elements in the cell's defense against ferroptosis. These insights broaden our comprehension of ferroptosis mechanisms and position kaempferol as a potential therapeutic candidate for drug development.

ABBREVIATIONS

ALOXs - Arachidonic Acid Lipoxygenases, **ANOVA** - Analysis of Variance, **ACSL4** - Acyl-CoA Synthetase Long-Chain Family Member 4, **CCK-8** - Cell Counting Kit-8, **DAPI** - 4',6-diamidino-2-phenylindole, **DMEM** - Dulbecco's Modified Eagle's Medium, **DHE** - Dihydroethidium, **FBS** - Fetal Bovine Serum, **GSH** - Glutathione, **GPX4** - Glutathione Peroxidase 4, **HO-1** - Heme Oxygenase-1, **HRP** - Horseradish Peroxidase, **HUVECs** - Human Umbilical Vein Endothelial Cells, **IRF** - Interferon Regulatory Factor, **LPCAT3** - Lysophosphatidylcholine Acyltransferase 3, **Nrf2** - Nuclear Factor Erythroid-Derived 2-Like 2, **PBS** - Phosphate-Buffered Saline, **PCs** - Procyanidins, **PVDF** - Polyvinylidene Difluoride, **qPCR** - Real-Time Fluorescence Quantitative Polymerase Chain Reaction, **RCD** - Regulated Cell Death, **RIPA** - Radioimmunoprecipitation Assay Buffer, **ROS** - Reactive Oxygen Species, **SD** - Standard Deviation, **SDS-PAGE** - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, **SLC7A11** - Solute Carrier Family 7 Member 11

ACKNOWLEDGMENTS

We would like to thank Editage (www.editage.cn) for English language editing.

AUTHOR'S CONTRIBUTIONS

All authors significantly contributed to this work, read and approved the final manuscript.

FUNDING

AVAILABILITY OF DATA AND MATERIALS

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

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Zhang H, Vreeken D, Bruikman CS, van Zonneveld AJ, van Gils JM. Understanding netrins and semaphorins in mature endothelial cell biology. Pharmacological Research. 2018;137:1–10. PMID: 30240825. Available from: https://doi.org/10.1016/j.phrs.2018.09.015.
2. Liu YV, Santiago CP, Sogunro A, Konar GJ, Hu MW, McNally MM. Single-cell transcriptome analysis of xenotransplanted human retinal organoids defines two migratory cell populations of nonretinal origin. Stem Cell Reports. 2023;18(5):1138–54. PMID: 37163980. Available from: https://doi.org/10.1016/j.stemcr.2023.04.004.
3. Salewskij K, Penninger JM. Blood Vessel Organoids for Development and Disease. Circulation Research. 2023;132(4):498–510. PMID: 36795852. Available from: https://doi.org/10.1161/CIRCRESAHA.122.321768.
4. Zheng D, Liu J, Piao H, Zhu Z, Wei R, Liu K. ROS-triggered endothelial cell death mechanisms: focus on pyroptosis, parthanatos, and ferroptosis. Frontiers in Immunology. 2022;13. PMID: 36389728. Available from: https://doi.org/10.3389/fimmu.2022.1039241.
5. Kulovic-Sissawo A, Tocantins C, Diniz MS, Weiss E, Steiner A, Tokic S. Mitochondrial Dysfunction in Endothelial Progenitor Cells: Unraveling Insights from Vascular Endothelial Cells. Biology (Basel). 2024;13(2):70. PMID: 38392289. Available from: https://doi.org/10.3390/biology13020070.
6. Liu Z, Zhang X, Xiong S, Huang S, Ding X, Xu M, et al. Endothelial dysfunction of syphilis: Pathogenesis. Journal of the European Academy of Dermatology and Venereology. 2024;2024:Early view. Available from: https://doi.org/10.1111/jdv.19899.
7. Mozzicato AM, Bastrup JA, Sanchez-Alonso JL, van der Horst J, Gorelik J, Hägglund P. Mesenteric artery smooth muscle cells from hypertensive rats have increased microtubule acetylation. The Biochemical Journal. 2024;481(5):387–403. PMID: 38373073. Available from: https://doi.org/10.1042/BCJ20230420.
8. Chen X, Kang R, Kroemer G, Tang D. Ferroptosis in infec-tion, inflammation, and immunity. The Journal of Experimen-tal Medicine. 2021;218(6). PMID: 33978684. Available from: https://doi.org/10.1084/jem.20210518.
9. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. Cell Research. 2019;29(5):347–64. PMID: 30948788. Available from: https: //doi.org/10.1038/s41422-019-0164-5.
10. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death and Differentiation. 2018;25(3):486–541. PMID: 29362479. Available from: https://doi.org/10.1038/s41418-017-0012-4.
11. Distéfano AM, Martin MV, Córdoba JP, Bellido AM, D'Ippólito S, Colman SL. Heat stress induces ferroptosis-like cell death in plants. The Journal of Cell Biology. 2017;216(2):463–76. PMID: 28100685. Available from: https://doi.org/10.1083/jcb.201605110.

12. Liu J, Kang R, Tang D. Signaling pathways and defense mechanisms of ferroptosis. The FEBS Journal. 2022;289(22):7038–50. PMID: 34092035. Available from: https://doi.org/10.1111/febs.16059.
13. Liu Y, Yang G, Huo S, Wu J, Ren P, Cao Y. Lutein suppresses fer-roptosis of cardiac microvascular endothelial cells via positive regulation of IRF in cardiac hypertrophy. European Journal of Pharmacology. 2023;959. PMID: 37797674. Available from: https://doi.org/10.1016/j.ejphar.2023.176081.
14. Chen L, Huang J, Yao ZM, Sun XR, Tong XH, Hu M. Procyanidins Alleviated Cerebral Ischemia/Reperfusion Injury by Inhibiting Ferroptosis via the Nrf2/HO-1 Signaling Pathway. Molecules (Basel, Switzerland). 2023;28(8):3582. PMID: 37110816. Available from: https://doi.org/10.3390/molecules28083582.
15. Xu WH, Li CH, Jiang TL. [Ferroptosis pathway and its intervention regulated by Chinese materia medica]. Zhongguo Zhongyao Zazhi. 2018;43(20):4019–26. PMID: 30486525.
16. Chagas MDSS, Behrens MD, Moragas-Tellis CJ, Penedo GX, Silva AR, Gonçalves-de Albuquerque CF. Flavonols and Flavones as Potential anti-Inflammatory, Antioxidant, and An-tibacterial Compounds. Oxidative Medicine and Cellular Longevity. 2022;2022:9966750. Available from: https://doi.org/10.1155/2022/9966750.
17. Muruganathan N, Dhanapal AR, Baskar V, Muthuramalingam P, Selvaraj D, Aara H. Recent Updates on Source, Biosynthesis, and Therapeutic Potential of Natural Flavonoid Luteolin: A Review. Metabolites. 2022;12(11):1145. PMID: 36422285. Available from: https://doi.org/10.3390/metabo12111145.
18. Liu L, Wang L, Xiao Y, Liu Y, Meng X, Shen X. Natural flavonoids act as potent ferroptosis inhibitors and their potentials in the treatment of ferroptosis-associated diseases. Pharmacolog-ical Research Modern Chinese Medicine. 2024;10. Available from: https://doi.org/10.1016/j.prmcm.2024.100377.
19. Periferakis A, Periferakis K, Badarau IA, Petran EM, Popa DC, Caruntu A, et al. Kaempferol: antimicrobial properties, sources, clinical, and traditional applications. International Journal of Molecular Sciences. 2022;23(23):15054. Available from: https://doi.org/10.3390/ijms232315054.
20. Alam W, Khan H, Shah MA, Cauli O, Saso L. Kaempferol as a Dietary Anti-Inflammatory Agent: Current Therapeutic Standing. Molecules (Basel, Switzerland). 2020;25(18):4073. PMID: 32906577. Available from: https://doi.org/10.3390/molecules25184073.
21. Akter M, Parvin MS, Hasan MM, Rahman MA, Islam ME. Anti-tumor and antioxidant activity of kaempferol-3-O-alpha-L-rhamnoside (Afzelin) isolated from Pithecellobium dulce leaves. BMC Complementary Medicine and Therapies. 2022;22(1):169. PMID: 35733130. Available from: https://doi.org/10.1186/s12906-022-03633-x.
22. Yang Y, Chen Z, Zhao X, Xie H, Du L, Gao H, et al. Mechanisms of Kaempferol in the treatment of diabetes: A comprehensive and latest review. Frontiers in endocrinology. 2022;13:990299. Available from: https://doi.org/10.3389/fendo.2022.990299.
23. Chen M, Xiao J, El-Seedi HR, Woźniak KS, Daglia M, Little PJ, et al. Kaempferol and atherosclerosis: From mechanism to medicine. Critical Reviews in Food Science and Nutri-tion. 2024;64(8):2157–2175. Available from: https://doi.org/10.1080/10408398.2022.2121261.
24. Liu H, Yi X, Tu S, Cheng C, Luo J. Kaempferol promotes BMSC osteogenic differentiation and improves osteoporo-sis by downregulating miR-10a-3p and upregulating CXCL12. Molecular and Cellular Endocrinology. 2021;520. PMID: 33157164. Available from: https://doi.org/10.1016/j.mce.2020. 111074.
25. Chang S, Li X, Zheng Y, Shi H, Zhang D, Jing B, et al. Kaempferol exerts a neuroprotective effect to reduce neuropathic pain through TLR4/NF-κ B signaling pathway. Phytotherapy Re-search. 2022;36(4):1678–91. PMID: 35234314. Available from: https://doi.org/10.1002/ptr.7396.
26. Xiao X, Hu Q, Deng X, Shi K, Zhang W, Jiang Y. Old wine in new bottles: kaempferol is a promising agent for treating the trilogy of liver diseases. Pharmacological Research. 2022;175. PMID: 34843960. Available from: https://doi.org/10.1016/j.phrs.2021.106005.
27. Kamisah Y, Jalil J, Yunos NM, Zainalabidin S. Cardioprotective Properties of Kaempferol: A Review. Plants. 2023;12(11):2096. PMID: 37299076. Available from: https://doi.org/10.3390/plants12112096.
28. Cheng X, Yang YL, Yang H, Wang YH, Du GH. Kaempferol alleviates LPS-induced neuroinflammation and BBB dysfunction in mice via inhibiting HMGB1 release and down-regulating TLR4/MyD88 pathway. International Immunopharmacology. 2018;56:29–35. PMID: 29328946. Available from: https://doi.org/10.1016/j.intimp.2018.01.002.
29. Li M, Zhang D, Zhang L, Wang LS, Tian Y, Li B. The inhibitory effect of chemical components from Ginkgo biloba flower on ferroptosis in vascular endothelial cells. Journal of International Pharmaceutical Research. 2020;47:857–62.
30. Gardiner B, Dougherty JA, Ponnalagu D, Singh H, Angelos M, Chen CA. Berliner LJ, Parinandi NL, editors. Measurement of Oxidative Stress Markers In Vitro Using Commercially Available Kits. Cham (CH): Springer; 2020. Available from: https: //doi.org/10.1007/978-3-030-47318-1_4.
31. Mei H, Zhao L, Li W, Zheng Z, Tang D, Lu X. Inhibition of ferroptosis protects House Ear Institute-Organ of Corti 1 cells and cochlear hair cells from cisplatin-induced ototoxicity. Journal of Cellular and Molecular Medicine. 2020;24(20):12065–81. PMID: 32929878. Available from: https://doi.org/10.1111/jcmm.15839.
32. Zhou D, Liang Q, Ge X, Xu J. Allogeneic platelet-rich plasma inhibits ferroptosis in promoting wound repair of type 2 diabetic ulcers. Free Radical Biology & Medicine. 2024;215:37–47. PMID: 38408545. Available from: https://doi.org/10.1016/j.freeradbiomed.2024.02.020.
33. Matsuda N, Imbaby S, Hattori K, Hattori Y. STAT3 decoy oligodeoxynucleotides improve end-organ tissue injury and survival in septic mice. The FASEB Journal. 2020;34:1–1. Avail-able from: https://doi.org/10.1096/fasebj.2020.34.s1.03240.
34. Imbaby S, Hattori Y. Stattic ameliorates the cecal ligation and puncture-induced cardiac injury in septic mice via IL-6-gp130-STAT3 signaling pathway. Life Sciences. 2023;330. PMID: 37549828. Available from: https://doi.org/10.1016/j.lfs.2023. 122008.
35. Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxidative Medicine and Cellular Longevity. 2019;2019. PMID: 31737171. Available from: https://doi.org/10.1155/2019/5080843.
36. Kapralov AA, Yang Q, Dar HH, Tyurina YY, Anthonymuthu TS, Kim R. Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. Nature Chemical Biology. 2020;16(3):278–90. PMID: 32080625. Avail-able from: https://doi.org/10.1038/s41589-019-0462-8.
37. Ferreira CA, Ni D, Rosenkrans ZT, Cai W. Scavenging of reactive oxygen and nitrogen species with nanomaterials. Nano Research. 2018;11(10):4955–84. PMID: 30450165. Available from: https://doi.org/10.1007/s12274-018-2092-y.
38. Baselet B, Sonveaux P, Baatout S, Aerts A. Pathological effects of ionizing radiation: endothelial activation and dysfunction. Cellular and Molecular Life Sciences. 2019;76(4):699–728. PMID: 30377700. Available from: https://doi.org/10.1007/s00018-018-2956-z.
39. Stockwell BR. Ferroptosis turns 10: emerging mechanisms, physiological functions, and therapeutic applications. Cell. 2022;185(14):2401–21. PMID: 35803244. Available from: https://doi.org/10.1016/j.cell.2022.06.003.
40. Dai Z, Zhang W, Zhou L, Huang J. Probing lipid peroxidation in ferroptosis: emphasizing the utilization of C11-BODIPY-based protocols. In: Ferroptosis: Methods and Protocols. New York, NY: Springer US, 2023. p. 61-72.
41. Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M. Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. eLife.

2014;3. PMID: 24844246. Available from: https://doi.org/10.7554/eLife.02523.
42. Campos J, Gleitze S, Hidalgo C, Núñez MT. IP3R-Mediated Calcium Release Promotes Ferroptotic Death in SH-SY5Y Neuroblastoma Cells. Antioxidants. 2024;13(2):196. PMID: 38397794. Available from: https://doi.org/10.3390/antiox13020196.
43. Schwantes A, Wickert A, Becker S, Baer PC, Weigert A, Brüne B. Tumor associated macrophages transfer ceruloplasmin mRNA to osarcoma cells and protect them from ferroptosis. Redox Biology. 2024;71. PMID: 38382185. Available from: https://doi.org/10.1016/j.redox.2024.103093.
44. Costa I, Barbosa DJ, Benfeito S, Silva V, Chavarria D, Borges F, et al. Molecular mechanisms of ferroptosis and their involvement in brain diseases. Pharmacology & Therapeutics. 2023;244. PMID: 36894028. Available from: https://doi.org/10.1016/j.pharmthera.2023.108373.
45. Wang L, Chen X, Yan C. Ferroptosis: an emerging therapeutic opportunity for cancer. Genes & Diseases. 2020;9(2):334–46. PMID: 35224150. Available from: https://doi.org/10.1016/j.gendis.2020.09.005.
46. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. Cell Research. 2021;31(2):107–25. PMID: 33268902. Available from: https://doi.org/10.1038/s41422-020-00441-1.
47. Li J, Cao F, Yin HL, Huang ZJ, Lin ZT, Mao N, et al. Ferroptosis: past, present and future. Cell Death & Disease. 2020;11(2):88. PMID: 32015325. Available from: https://doi.org/10.1038/s41419-020-2298-2.
48. Ma X, Zhang X, Wang X, Wang C, Ma Y. The role of kaempferol in gynaecological malignancies: progress and perspectives. Frontiers in Pharmacology. 2023;14. PMID: 38143502. Available from: https://doi.org/10.3389/fphar.2023.1310416.
49. Wu P, Meng X, Zheng H, Zeng Q, Chen T, Wang W. Kaempferol Attenuates ROS-Induced Hemolysis and the Molecular Mechanism of Its Induction of Apoptosis on Bladder Cancer. Molecules (Basel, Switzerland). 2018;23(10):2592. PMID: 30309003. Available from: https://doi.org/10.3390/molecules23102592.
50. Yuan Y, Zhai Y, Chen J, Xu X, Wang H. Kaempferol Ameliorates Oxygen-Glucose Deprivation/Reoxygenation-Induced Neuronal Ferroptosis by Activating Nrf2/SLC7A11/GPX4 Axis. Biomolecules. 2021;11(7):923. PMID: 34206421. Available from: https://doi.org/10.3390/biom11070923.
51. Li H, Weng Q, Gong S, Zhang W, Wang J, Huang Y, et al. Kaempferol prevents acetaminophen-induced liver injury by suppressing hepatocyte ferroptosis via Nrf2 pathway activation. Food & Function. 2023;14(4):1884–96. PMID: 36723004. Available from: https://doi.org/10.1039/D2FO02716J.
52. Dong X, Zhou S, Nao J. Kaempferol as a therapeutic agent in Alzheimer's disease: evidence from preclinical studies. Ageing Research Reviews. 2023;87. PMID: 36924572. Available from: https://doi.org/10.1016/j.arr.2023.101910.
53. Wang Y, Wu J. Ferroptosis: a new strategy for cardiovascular disease. Frontiers in Cardiovascular Medicine. 2023;10. PMID: 37731525. Available from: https://doi.org/10.3389/fcvm.2023.1241282.
54. Lou X, Zhang Y, Guo J, Gao L, Ding Y, Zhuo X. What is the impact of ferroptosis on diabetic cardiomyopathy: a systematic review. Heart Failure Reviews. 2023;29(1):1–11. PMID: 37555989. Available from: https://doi.org/10.1007/s10741-023-10336-z.
55. Xu X, Xu XD, Ma MQ, Liang Y, Cai YB, Zhu ZX. The mechanisms of ferroptosis and its role in atherosclerosis. Biomedicine and Pharmacotherapy. 2024;171. PMID: 38171246. Available from: https://doi.org/10.1016/j.biopha.2023.116112.
56. Koeberle SC, Kipp AP, Stuppner H, Koeberle A. Ferroptosis-modulating small molecules for targeting drug-resistant cancer: challenges and opportunities in manipulating redox signaling. Medicinal Research Reviews. 2023;43(3):614–82. PMID: 36658724. Available from: https://doi.org/10.1002/med.21933.
57. Fu P, Chen Y, Wu M, Bao B, Yin X, Chen Z. Effect of ferroptosis on chronic cerebral hypoperfusion in vascular dementia. Experimental Neurology. 2023;370. PMID: 37709116. Available from: https://doi.org/10.1016/j.expneurol.2023.114538.
58. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. Nature Reviews Molecular Cell Biology. 2021;22(4):266–82. PMID: 33495651. Available from: https://doi.org/10.1038/s41580-020-00324-8.
59. Cartland SP, Stanley CP, Bursill C, Passam F, Figtree GA, Patel S. Sex, Endothelial Cell Functions, and Peripheral Artery Disease. International Journal of Molecular Sciences. 2023;24(24):17439. PMID: 38139267. Available from: https: //doi.org/10.3390/ijms242417439.
60. Jung JM, Gruber A, Heseltine P, Rajamani K, Ameriso SF, Fisher MJ. New Directions in Infection-Associated Ischemic Stroke. Journal of Clinical Neurology (Seoul, Korea). 2024;20(2):140–52. PMID: 38330416. Available from: https://doi.org/10.3988/jcn.2023.0056.
61. Yubero-Serrano EM, Fernandez-Gandara C, Garcia-Rios A, Rangel-Zuñiga OA, Gutierrez-Mariscal FM, Torres-Peña JD. Mediterranean diet and endothelial function in patients with coronary heart disease: an analysis of the CORDIOPREV randomized controlled trial. PLoS Medicine. 2020;17(9). PMID: 32903262. Available from: https://doi.org/10.1371/journal.pmed.1003282.
62. Yin J, Fu X, Luo Y, Leng Y, Ao L, Xie C. Narrative Review of Diabetic Macroangiopathy: From Molecular Mechanism to Therapeutic Approaches. Diabetes Therapy. 2024;15:585–609. Available from: https://doi.org/10.1007/s13300-024-01532-7.
63. Bartimoccia S, Praktiknjo M, Nocella C, Schierwagen R, Cammisotto V, Jansen C. Association between endotoxemia and blood no in the portal circulation of cirrhotic patients: results of a pilot study. Internal and Emergency Medicine. 2024;19(3):713–20. PMID: 38409619. Available from: https: //doi.org/10.1007/s11739-024-03534-6.
64. Díaz-Flores L, Gutiérrez R, González-Gómez M, García MD, Carrasco-Juan JL, Martín-Vasallo P. Phenomena of Intussusceptive Angiogenesis and Intussusceptive Lymphangiogenesis in Blood and Lymphatic Vessel Tumors. Biomedicines. 2024;12(2):258. PMID: 38397861. Available from: https://doi.org/10.3390/biomedicines12020258.
65. Zhou J, Zhou P, Wang J, Song J. Roles of endothelial cell specific molecule 1 in tumor angiogenesis (Review). Oncology Letters. 2024;27(3):137. PMID: 38357478. Available from: https://doi.org/10.3892/ol.2024.14270.
66. Luo H, Jiang BH, King SM, Chen YC. Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids. Nu-trition and Cancer. 2008;60(6):800–9. PMID: 19005980. Avail-able from: https://doi.org/10.1080/01635580802100851.
67. Bangar SP, Chaudhary V, Sharma N, Bansal V, Ozogul F, Lorenzo JM. Kaempferol: A flavonoid with wider biological activities and its applications. Critical Reviews in Food Science and Nutrition. 2023;63(28):9580–604. PMID: 35468008. Available from: https://doi.org/10.1080/10408398.2022.2067121.