

Mechanism of Action of Raspberry Natural Extract on Apoptosis in Human Brain Artery Aneurysm Vascular Endothelial Cells

Pharmacognosy Magazine

22(1) 265–276, 2026

© The Author(s) 2025

Article reuse guidelines:

in.sagepub.com/journals-permissions-india

DOI: 10.1177/09731296251321210

journals.sagepub.com/home/phm

Junti Lu^{1,2}, Yanhui Zhou³, Zhenyu Xia², Bowen Yang², Gao Wu², Hui Gui²
and Shiwen Guo¹ 

Abstract

Background and Objectives: Raspberry natural extract exhibits a regulatory effect on apoptosis in human brain artery aneurysm vascular endothelial cells (VECs). This work was to gain deeper insights into the impact of raspberry extract on apoptosis in VECs in the treatment of human brain artery aneurysms, along with the associated molecular mechanisms, thereby providing scientific support for its application in the treatment of human brain artery aneurysms.

Materials and Methods: High-performance liquid chromatography was employed to determine the primary components of raspberry extract. Human brain artery aneurysm VECs were divided into the following groups: model (M) group, raspberry low-dose (LD) group (50 µg/mL), raspberry medium-dose (MD) group (100 µg/mL), raspberry high-dose (HD) group (150 µg/mL), and normal human brain artery VECs as the control (C) group. Cell viability was assessed under 3-(4,5)-dimethylthiazolyl (2,5)-phenyltetrazolium bromide assay. Levels of relevant proteins were measured under Western blot, and related indicators were evaluated in each group.

Results: Raspberry extract included ellagic acid, rutin, kaempferol-3-O-rutinoside, oleanolic acid, tiliroside, quercetin, and kaempferol. The cell viability of brain VECs in the raspberry extract groups was drastically superior to that in the M group ($p < 0.05$). In the HD group, cell viability of brain VECs was drastically superior to that in the LD group ($p < 0.05$). Brain VECs in the raspberry extract groups exhibited greatly inferior levels of matrix metalloproteinase-9 (MMP-9), interleukin (IL)-17A, and endothelin (ET)-1 to M group ($p < 0.05$). In the HD group, brain VECs showed notably lower levels of MMP-9, IL-17A, and ET-1 versus the LD group ($p < 0.05$). PI3K and AKT in brain VECs were substantially superior in the raspberry extract groups to the M group ($p < 0.05$). In HD group, levels of Bax, Caspase8, Cleaved-caspase8, Caspase12, Cleaved-caspase12, Caspase3, and Cleaved-caspase3 in brain VECs were markedly inferior to those in LD group ($p < 0.05$), while Bcl-2 level in HD group was drastically superior to LD group ($p < 0.05$).

Conclusion: Raspberry natural extract can enhance the survival rate of brain VECs, suppress cell apoptosis, and protect VECs. Raspberry extract can reduce inflammatory factors in brain VECs, activate PI3K/AKT signaling, and modulate apoptosis-related protein levels.

Keywords

Raspberry, human brain artery aneurysm, vascular endothelial cells, cell apoptosis, PI3K/AKT signaling

Received 25 September 2024; accepted 16 December 2024

Introduction

The primary characteristic of cerebral artery aneurysms is the localized dilation of the arterial wall, which accelerates blood flow within the affected area, resulting in increased pressure on the aneurysm wall and subsequently causing chronic or acute damage to brain tissue (Brie et al., 2024). Studies have indicated that cerebrovascular diseases are a leading cause of

¹Department of Neurosurgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China

²Department of Neurosurgery, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China

³Department of Pulmonary and Critical Care Medicine, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China

Corresponding author:

Shiwen Guo, Department of Neurosurgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China.

E-mail: guosiwen2023@163.com



vascular ruptures in individuals, posing a significant risk of disability and mortality (Han et al., 2016; Li et al., 2023). The mechanisms underlying the occurrence and progression of cerebral artery aneurysms have been a long-standing focus of research, with multiple factors showing significant associations with their occurrence (Hua et al., 2023).

The role of natural raspberry extract in the apoptosis of human brain artery aneurysm vascular endothelial cells (VECs) has long been a focus of researchers' attention (Ke et al., 2019; Kong et al., 2022). Raspberry extract may have mechanisms that confer anti-apoptotic effects and protect the functionality of VECs, potentially becoming a prospective drug for treating human brain artery aneurysms (Ke et al., 2021; Wu et al., 2022). VECs play a critical role in maintaining vascular structure and function (Jin et al., 2023). Studies have shown that abnormal apoptosis of VECs is more prominent in the lesion sites of human brain artery aneurysms (Wang et al., 2024). Apoptosis is an important pathway for normal cell death, and it regulates development, tissue recovery, and cellular metabolism (Chen et al., 2021). Excessive apoptosis of VECs may lead to damage to the vascular endothelial barrier function, thereby triggering the occurrence and progression of aneurysms (Zhang et al., 2017; Zhong et al., 2022). Therefore, finding drugs and treatment methodologies that can regulate VEC apoptosis is of great significance for exploring the pathophysiological mechanisms of human brain artery aneurysms and seeking new treatment strategies. Raspberry natural extract is rich in polyphenolic compounds, such as flavonoids and anthocyanins, which are related to various physiological processes, including anti-tumor, anti-thrombotic, and anti-cardiovascular diseases (Zhang et al., 2022). Furthermore, raspberry extract is also abundant in nutritional components such as vitamin C, vitamin E, and β -carotene, which can protect VECs from oxidative stress damage. Studies have indicated that raspberry extract can modulate cell apoptosis through multiple pathways, mitigating the extent of apoptosis in VECs (Wang et al., 2023). On one hand, raspberry extract can influence VEC apoptosis by regulating apoptosis-related factors (Chen et al., 2019). Research has revealed that raspberry extract can downregulate the expression of certain pro-apoptotic factors (Bax, Caspase-9, and Caspase-3) while upregulating anti-apoptotic factor levels such as Bcl-2 (Ding, 2011). The regulation of these molecules may be mediated through the abundant polyphenolic compounds present in raspberry extract. Polyphenolic compounds possess significant antioxidant capabilities, inhibiting intracellular free radical generation and oxidative stress, thereby reducing cell apoptosis (Hsieh et al., 2002). On the other hand, raspberry extract can also protect VECs by activating cell survival pathways (Luo et al., 2023). Research has demonstrated that raspberry extract can activate PI3K/AKT and MAPK pathways, which are involved in apoptosis. By inhibiting apoptosis-related proteins, these pathways enhance cell survival (Lei et al., 2023). Furthermore, raspberry extract can also influence the progression of the cell

cycle, further suppressing apoptosis in VECs. Raspberry natural extract exhibits a significant regulatory effect on apoptosis in human brain artery aneurysm VECs (Xu et al., 2021). Its antioxidant and anti-inflammatory properties, along with its modulation of apoptosis-related factors, are likely the primary mechanisms of its action. A reference has been provided for further exploring the pathogenesis of human brain artery aneurysms and developing new treatment approaches, while also laying a theoretical foundation for the clinical application of raspberry extract (Zhang et al., 2019). Nevertheless, further experimental research and clinical trials are still needed for assessment of its efficacy and safety, so as to provide more effective drug strategies for the treatment of human brain artery aneurysms.

This work aimed to investigate the molecular, genetic, and cellular factors involved in the pathogenesis of human brain artery aneurysms. By systematically integrating experimental data from various sources and combining the results of animal experiments and clinical observations, it aimed to discuss the mechanism of different molecules and pathways in the progression of human brain artery aneurysms.

Materials and Methods

Preparation and Component Analysis of Raspberry Extract

High-performance liquid chromatography (HPLC; S3000, Qingdao Juchuang Environmental Protection Group Co., Ltd., China) was employed to determine the major components of raspberry extract. Precisely weighed 0.5 g of raspberry herbal powder (Bozhou Huozhentang Pharmaceutical Co., Ltd., China) was added to 50 mL of 70% methanol, sonicated for dissolution, and then subjected to a 3-h extraction at 68°C. Subsequently, the solution was cooled to 25°C and filtered to obtain the test sample solution. For the reference standards (Sigma-Aldrich, USA), ellagic acid, kaempferol-3-*O*-rutinoside, tiliroside, kaempferol, quercetin, rutin, and oleanolic acid were precisely weighed and dissolved in methanol to 25 mL. These reference solutions were also sonicated for dissolution and subjected to a 3-h extraction at 68°C, then cooling to 25°C and filtration to obtain the reference standard solutions. The chromatographic column used was a Thermo ODS C₁₈ column (250 × 4.6 mm, 5 μ m). The mobile phase included acetonitrile and 0.2% phosphoric acid aqueous solution under 1 mL/min. Detection wavelengths were set at 254 nm (ellagic acid; Sanofi Pharmaceutical Co., Ltd., Zhejiang, China) from 0 to 8 min, 344 nm (rutin, oleanolic acid, and kaempferol-3-*O*-rutinoside; Sanofi Pharmaceutical Co., Ltd., Zhejiang, China) from 8 to 39 min, 316 nm (tiliroside; Sanofi Pharmaceutical Co., Ltd., Zhejiang, China) from 39 to 45 min, and 254 nm (quercetin and kaempferol; Sanofi Pharmaceutical Co., Ltd., Zhejiang, China) from 45 to 60 min. The column temperature was maintained at 30°C with 20 μ L injection volume.

Cell Grouping and Treatment

Human brain artery aneurysm VECs (Shanghai Jinyuan Biotechnology Co., Ltd., China) were obtained and divided into the following groups: model (M) group, raspberry low-dose (LD) group (50 $\mu\text{g/mL}$), raspberry medium-dose (MD) group (100 $\mu\text{g/mL}$), and raspberry high-dose (HD) group (150 $\mu\text{g/mL}$). Moreover, normal human brain artery VECs were regarded as the control (C) group. In the M group, cells were cultured routinely without any drug treatment. In the raspberry LD group, raspberry extract was applied at 50 $\mu\text{g/mL}$. In the raspberry MD group, raspberry extract was 100 $\mu\text{g/mL}$. In the raspberry HD group, raspberry extract was 150 $\mu\text{g/mL}$. After drug administration, cells in all groups were further cultured for 24 h. The cell experiment process is shown in Figure 1.

3-(4,5)-Dimethylthiazoliazoyl-3,5-di-phenyltetrazoliumromide (MTT) Assay for Detecting Cell Survival Rate

Human brain artery VECs (logarithmic growth phase) from each group were routinely seeded into a 96-well plate. Following 24-h cultivation, the original medium was discarded, and the cells were grouped and treated. Subsequently, under the specification of the MTT cell proliferation assay kit (C0009S, Shanghai Beyotime Biotechnology Co., Ltd., China), cells were further cultured for 12, 24, 48, and 72 h,

and 10 μL of 5 mg mL^{-1} MTT reagent was applied and incubated for 4 h. Afterward, 150 μL of dimethyl sulfoxide (DMSO, 506008, Sigma-Aldrich, USA) was applied to each well, mixed thoroughly in the dark using a shaker for 10 min, and absorbance (A value) was measured employing a multifunctional microplate reader (SpectraMax Mini, Shanghai Meigu Molecular Devices Co., Ltd., China) to determine cell viability.

Detection of Cell-related Indicators

The levels of cell supernatant metalloproteinase-9 (MMP-9), interleukin (IL)-17A, and endothelin (ET)-1 were determined as follows. Control samples, test samples, and detection antibodies labeled with HRP were applied and incubated at 37°C for 1 h. After decanting and plate washing 5 times using a plate washer, 50 μL of substrate was applied, and the plate was incubated in the dark at 37°C for 15 min. Subsequently, 50 μL of stop solution was utilized to terminate the color reaction. Absorbance values at 450 nm were measured with a multifunctional microplate reader (SpectraMax Mini, Shanghai Meigu Molecular Devices Co., Ltd., China), and levels of MMP-9, IL-17A, and ET-1 in the cell supernatant of each group were calculated. MMP-9 and ET-1 detection kits were purchased from Beijing Biolab Technology Co., Ltd. (ZN2330, ZN2156, China). IL-17A detection kits were purchased from Shanghai Tuoyang Biotechnology Co., Ltd. (HA004917, China).

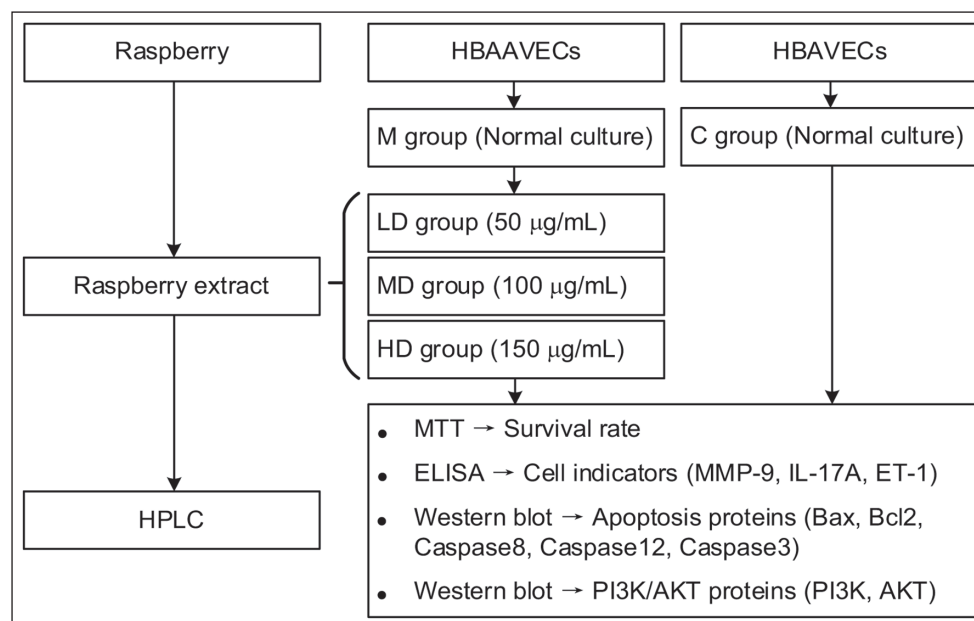


Figure 1. Experimental Flowchart.

Note: ELISA: Enzyme-linked immunosorbent assay; ET-1: Endothelin-1; IL-17A: Interleukin-17A; HBAVECs: Human brain artery aneurysm vascular endothelial cells; HBAVECs: Human brain artery vascular endothelial cells; HPLC: High-performance liquid chromatography; MMP-9: Matrix metalloproteinase-9; MTT: 3-(4,5)-Dimethylthiazoliazoyl-3,5-di-phenyltetrazoliumromide.

Western Blotting

Human brain artery VECs were initially seeded in 6-well plates and cultured for 48 h. Post-culturing, cells were harvested, and total protein extraction was performed through cell lysis using radioimmunoprecipitation assay lysis buffer (R0010, Solarbio, USA), followed by protein quantification using a bicinchoninic acid (BCA) assay kit. Protein separation was achieved via 10% SDS-PAGE gel electrophoresis and subsequent transfer onto a nitrocellulose membrane. The nitrocellulose membrane was blocked with 5% skim milk at 25°C for 2 h, and 1:2,000 diluted Bax, Bcl-2, Caspase8, Cleaved-caspase8, Caspase12, Cleaved-caspase12, Caspase3, Cleaved-caspase3, and β -actin primary antibodies (ab32503, ab182858, ab108333, ab61755, ab315271, ab8118, ab32351, ab32042, ab8226, Abcam, UK) were incubated overnight at 4°C. The membrane underwent triple rinsing with Tris-hydrochloric acid buffer solution (TBST) (B1009, Guizhou Yibai Pharmaceutical Co., Ltd., China) and a 2-h incubation with 1:5,000 diluted horseradish peroxidase-conjugated IgG secondary antibodies (ab6759, Abcam, UK). After another three TBST rinses, the membrane was subjected to a 5-min incubation in the dark with enhanced chemiluminescence (ECL) chemiluminescent reagent (34075, Thermo Fisher, USA), followed by development and exposure using the ECL chemiluminescence reagent. Under the gel imaging system (WD-9413A) (Beijing 61 Biological Technology Co., Ltd., China), ImageJ was utilized to quantify the relative grayscale intensities of the protein bands.

Statistical Analysis

Statistical analysis was conducted on all experimental data under SPSS 19.0, and results were denoted as Mean $\pm$ Standard Deviation. Comparisons between two independent samples were performed with Student's *t*-test, while differences among multiple groups were assessed using one-way analysis of variance (ANOVA). Statistical significance was denoted at *p* less than 0.05.

Results

Raspberry Components

Figure 2A–H depicts the HPLC chromatogram of raspberry extract, revealing that the major components of raspberries include ellagic acid, rutin, kaempferol-3-*O*-rutoside, oleanolic acid, tiliroside, quercetin, and kaempferol.

Effect of Raspberry Extract on the Survival Rate of Brain VECs

Figure 3 illustrates the impact of raspberry extract on the survival rate of brain VECs. The survival rate of brain VECs in the raspberry extract groups was drastically superior to that in the M group (*p* < 0.05). HD group displays a notably superior survival rate to the LD group (*p* < 0.05).

Influence of Raspberry Extract on Cell Supernatant Indicators of Human Cerebral Aneurysm VECs

Figure 4A–C illustrates the impact of the raspberry extract on the cell supernatant markers of human cerebral artery aneurysm endothelial cells. It was evident that in the raspberry extract group, MMP-9, IL-17A, and ET-1 in cerebral artery endothelial cells were markedly inferior to those in the M group (*p* < 0.05). Furthermore, in the HD group, levels of MMP-9, IL-17A, and ET-1 in cerebral artery endothelial cells were markedly inferior to those in the LD group (*p* < 0.05).

Impact of Raspberry Extract on PI3K/AKT Pathway in Human Cerebral Aneurysm VECs

Figure 5A–E illustrates the impact of the raspberry extract on the PI3K/AKT pathway in human cerebral artery aneurysm endothelial cells. It was evident that PI3K and AKT in raspberry extract groups were drastically superior to the M group (*p* < 0.05).

Influence of the Raspberry Extract on the Protein Level of VECs in Human Cerebral Aneurysms

Figure 6A–I depicts the impact of the raspberry extract on the protein levels in human cerebral artery aneurysm endothelial cells. In the HD group, the levels of Bax, Caspase8, Cleaved-caspase8, Caspase12, Cleaved-caspase12, Caspase3, and Cleaved-caspase3 in cerebral artery endothelial cells were markedly lower compared to the LD group (*p* < 0.05). In HD group, the level of Bcl2 in cerebral artery endothelial cells was markedly higher compared to LD group (*p* < 0.05).

Discussion

In this work, the main components were extracted from raspberries, revealing that the primary constituents are ellagic acid, rutin, and kaempferol-3-*O*-rutoside, among others. Studies have indicated that the antioxidant components of raspberries, such as ellagic acid, rutin, and quercetin, may have the potential to reduce cell apoptosis (Xu et al., 2023). These compounds can reduce apoptosis by increasing cellular antioxidant capacity and decreasing the production of oxidative stress and intracellular reactive oxygen species. Furthermore, oleanolic acid has also been reported to possess anti-inflammatory and cytoprotective properties, which may have a positive impact on the apoptosis of VECs. Kaempferol is a major component in raspberries known for its antioxidant and anti-inflammatory characteristics. Nevertheless, research on the effects of kaempferol on apoptosis in human brain artery aneurysm VECs remains relatively limited. In experiments involving human brain artery endothelial cells treated with hydrogen peroxide (H₂O₂), kaempferol greatly reduced apoptosis rate and decreased intracellular reactive oxygen

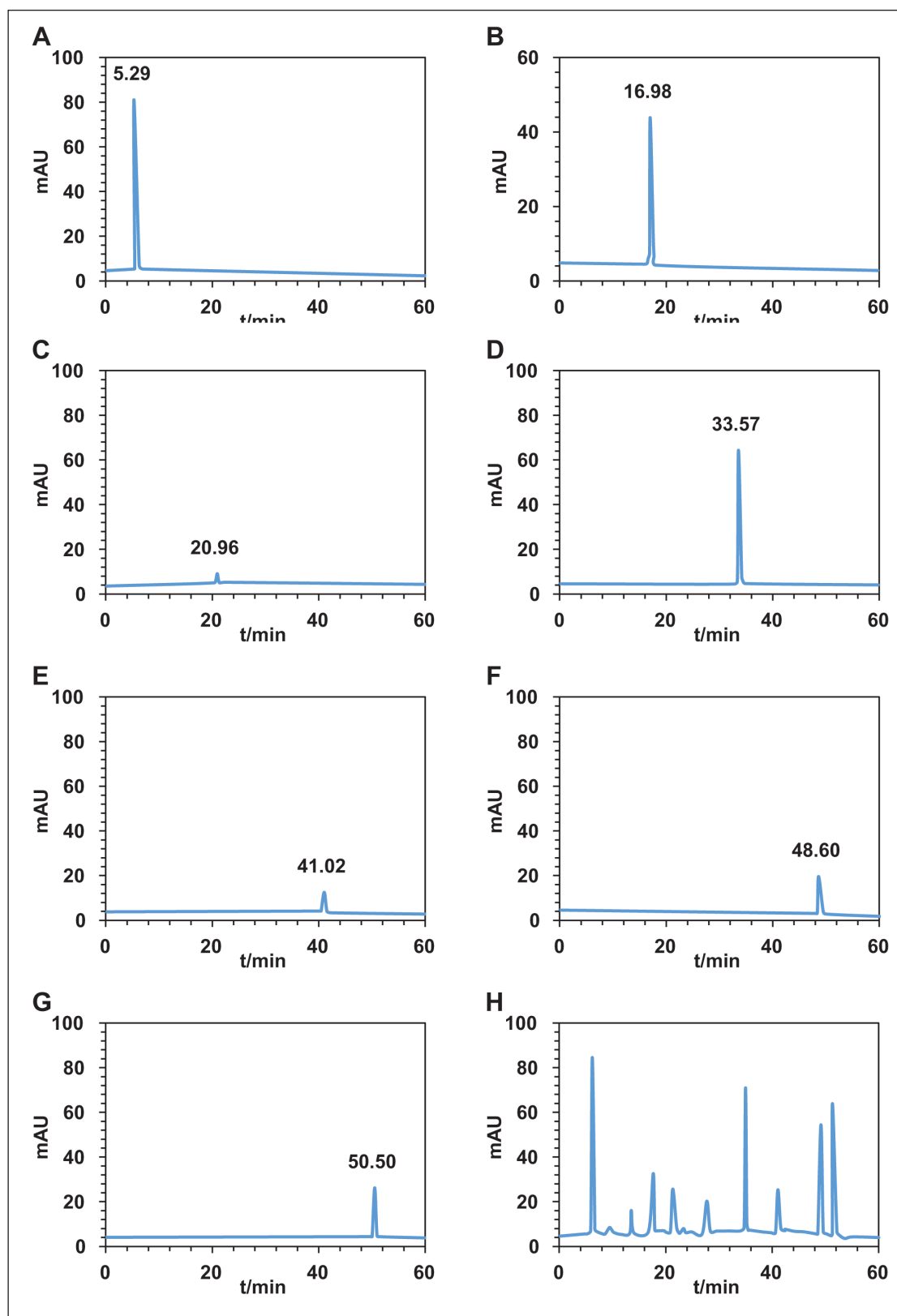


Figure 2. High-performance Liquid Chromatography (HPLC) Chromatogram of Raspberry. (A) Ellagic Acid; (B) Rutin; (C) Kaempferol-3-O-rutinoside; (D) Oleanolic Acid; (E) Tiliroside; (F) Quercetin; (G) Kaempferol; (H) the Test Sample of Raspberry.

species. These findings suggest that kaempferol may protect VECs from apoptosis-induced damage by reducing oxidative stress and intracellular reactive oxygen species (Li et al., 2021). Kaempferol also modulates Wnt/ β -catenin signaling, inhibiting its activation and suppressing the production of

inflammatory factors and cell apoptosis. This further underscores the potential of kaempferol in anti-inflammatory and anti-apoptotic activities (He et al., 2020). The raspberries' components may have certain potential in regulating apoptosis in VECs. Nevertheless, further research is needed to validate and thoroughly analyze the relevant effects and mechanisms.

Subsequently, this study evaluated the effects of raspberry extract on human brain microvascular endothelial cells (HBMECs), finding that it effectively enhances cell viability and exhibits concentration-dependent characteristics. Raspberries contain abundant flavonoid compounds, polyphenols, anthocyanins, and other components, all possessing robust antioxidant capabilities (Jiang et al., 2021). Oxidative stress is one of the primary factors leading to damage and apoptosis in brain VECs, and antioxidant components can effectively shield cells from oxidative stress damage. The HD group contains a higher concentration of antioxidant components, providing more effective protection against oxidative stress-induced damage and promoting cell survival. Furthermore, the HD group demonstrates superior anti-inflammatory capabilities. Inflammation is another significant factor inducing apoptosis in brain VECs (Wan et al., 2021). The HD group contains a higher concentration of

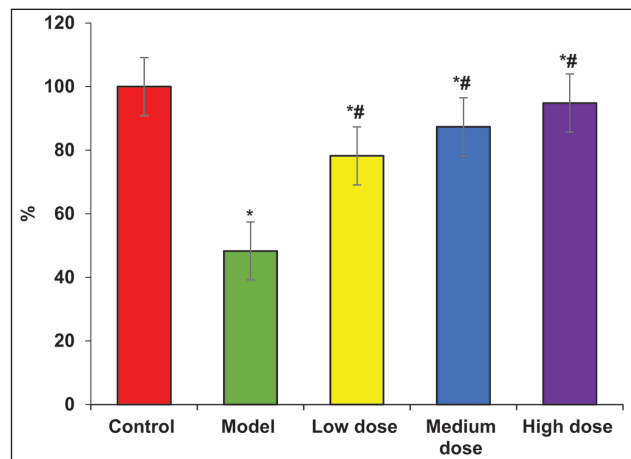


Figure 3. Effect of Raspberry Extract on the Survival Rate of Cerebral Vascular Endothelial Cells (VECs).

Note: * $p < 0.05$ versus C group; # $p < 0.05$ versus M group.

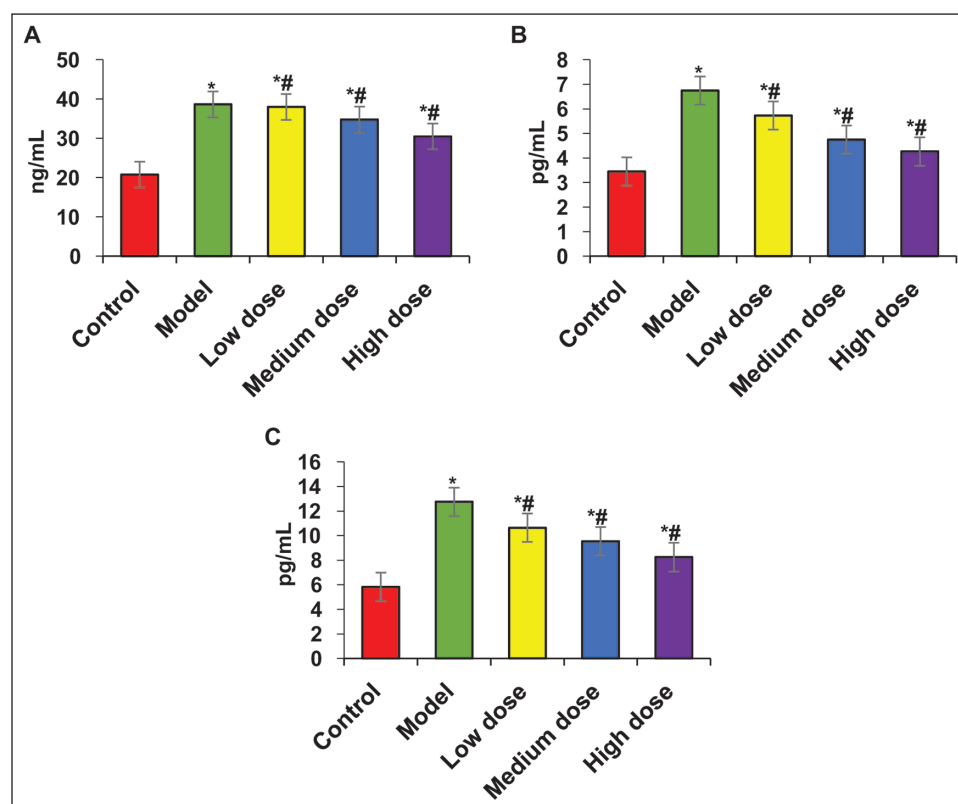


Figure 4. Influence of Raspberry Extract on Cell Supernatant Indicators of Endothelial Cells in Human Cerebral Aneurysms. A Shows Matrix Metalloproteinase-9 (MMP-9); B Shows Interleukin-17A (IL-17A); C Shows Endothelin-I (ET-I).

Note: * $p < 0.05$ versus C group; # $p < 0.05$ versus M group.

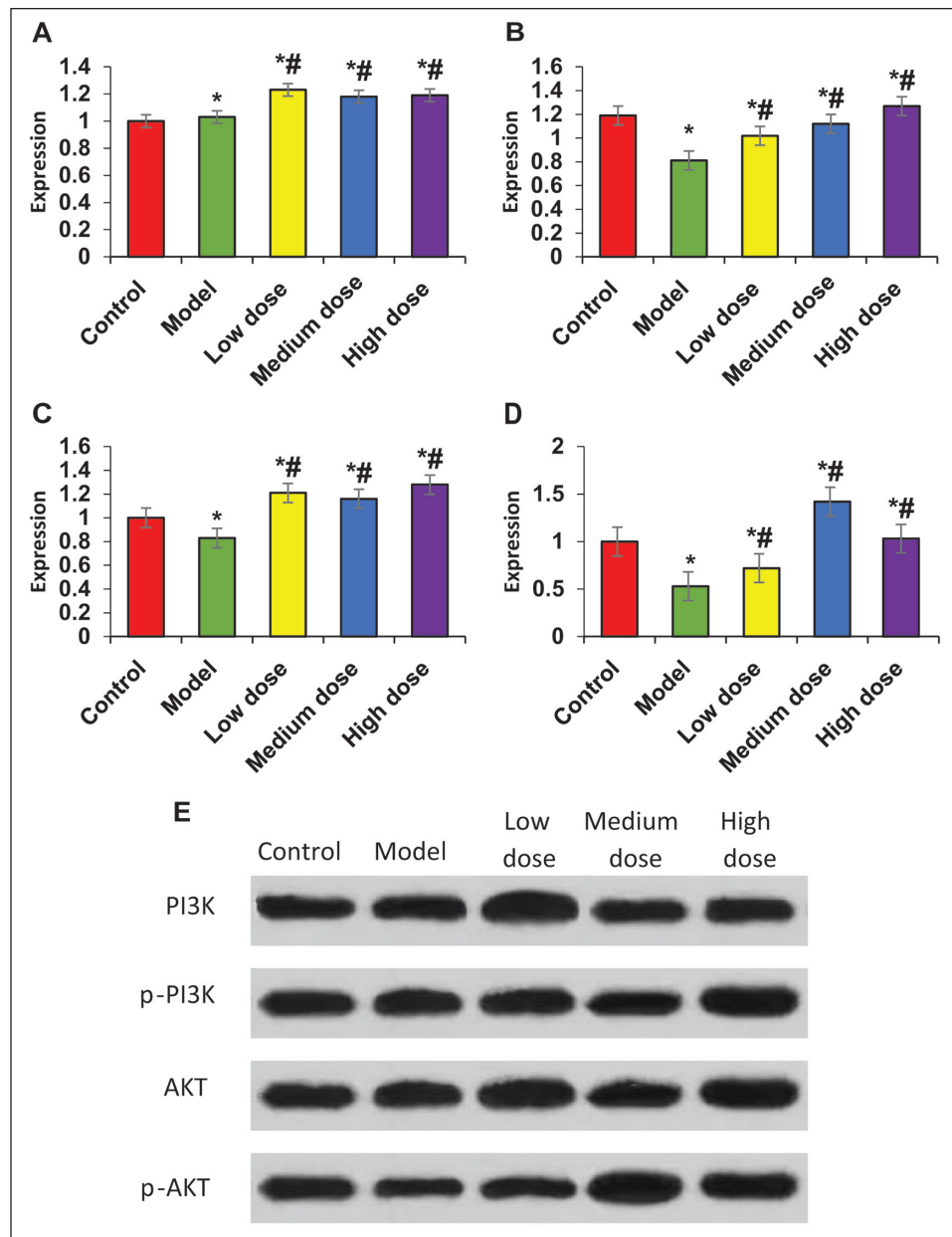


Figure 5. Impact of Raspberry Extract on PI3K/AKT Pathway in Endothelial Cells of Human Cerebral Aneurysms. (A) PI3K; (B) p-PI3K; (C) AKT; (D) p-AKT; (E) Results of Protein Blot Detection.

Note: * $p < 0.05$ versus C group; # $p < 0.05$ versus M group.

anti-inflammatory components, which can more effectively suppress the inflammatory response, reducing cell damage and apoptosis (Zhang et al., 2015). Consequently, the survival rate of brain VECs in the HD group is drastically superior to that in the LD and M groups. It can shield cells more effectively from oxidative stress, inflammatory responses, and nutrient deficiencies, promoting their growth and survival. This holds significance when considering raspberry extract as a therapeutic approach for preventing endothelial cell apoptosis. Optimizing the bioactivity and dosage of raspberry extract can enhance its therapeutic effectiveness. Furthermore,

this investigation found that raspberry extract can inhibit MMP-9, IL-17A, and ET-1 levels in HBMECs. MMP-9 is a matrix metalloproteinase that is upregulated in inflammatory and tumor environments, capable of degrading the extracellular matrix and promoting pathological vascular remodeling. Raspberry extract modulates immune responses and inflammatory processes, reducing the synthesis and release of IL-17A (Zhong et al., 2015). ET-1 is a peptide molecule of the endothelin family and acts crucially in the cardiovascular system. Reducing ET-1 levels can alleviate vascular constriction, inhibit thrombus formation, and improve vascular function,

which is paramount for preventing and treating cerebral artery aneurysms (Chen et al., 2020). The ability of raspberry extract to lower levels of MMP-9, IL-17A, and ET-1 is primarily attributed to its diverse bioactive components, which can regulate cellular pathways and gene expression, suppress inflammatory responses, and mitigate vascular damage.

PI3K/AKT pathway is an important cellular signaling pathway involved in various biological processes such as cell proliferation, survival, migration, and apoptosis. It is also crucial in inflammation and immune regulation. This study found significant changes in PI3K/AKT pathway-related protein expression in HBMECs after treatment with raspberry extract. This suggests that raspberry extract may regulate the PI3K/AKT pathway to inhibit abnormal proliferation and invasion of endothelial cells, thereby reducing vascular damage and preventing the occurrence of cerebral aneurysms. In addition to directly inhibiting activation of the PI3K/AKT pathway, raspberry extract may also affect endothelial cell function by regulating the expression and activity of downstream targets (Cyboran-Mikołajczyk et al., 2022). Raspberry extract's antioxidants can enhance cellular antioxidant capacity, alleviate oxidative damage to endothelial cells, and improve vascular function (Zeng et al., 2018). Raspberry extract may regulate the PI3K/AKT signaling pathway to modulate the release of inflammatory factors and the activity

of immune cells, thereby reducing inflammation and immune responses and alleviating damage to VECs (Chou et al., 1987; Zan et al., 2018). Bax protein is an important regulatory factor in the apoptosis signaling pathway, promoting the release of cytochrome C from mitochondria into cytoplasm, subsequently activating Caspase8 and Caspase9 proteins. This study found significant changes in the expression of Bax, Bcl-2, Caspase8, Caspase12, and Caspase3 proteins in HBMECs after treatment with raspberry extract. This indicates that raspberry extract may reduce activation of the apoptosis signaling pathway by inhibiting Bax expression and regulating Caspase protein activity, thereby reducing levels of Bax, Caspase8, Caspase12, and Caspase3 (Yau et al., 2002). Oxidative damage and inflammatory reactions are important factors leading to cell apoptosis. Raspberry extract may inhibit oxidative stress and inflammatory reactions, thereby reducing Caspase activation and Bax protein expression, and subsequently lowering levels of Cleaved-caspase8, Cleaved-caspase12, and Cleaved-caspase3. As a natural plant extract, raspberry extract has the ability to lower levels of apoptosis-related proteins, potentially playing a significant role in the prevention and treatment of vascular diseases such as cerebral aneurysms (Yang et al., 2022). By reducing levels of Bax, Caspase8, Cleaved-caspase8, Caspase12, Cleaved-caspase12, Caspase3, and Cleaved-caspase3, raspberry

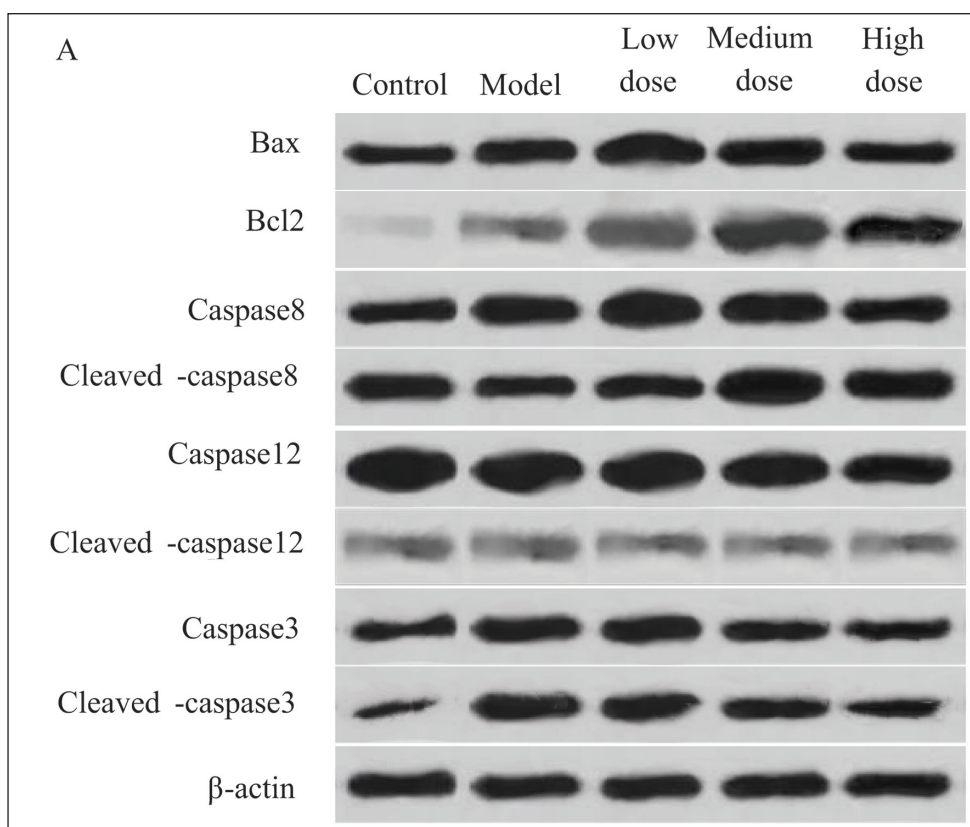


Figure 6. (continued)

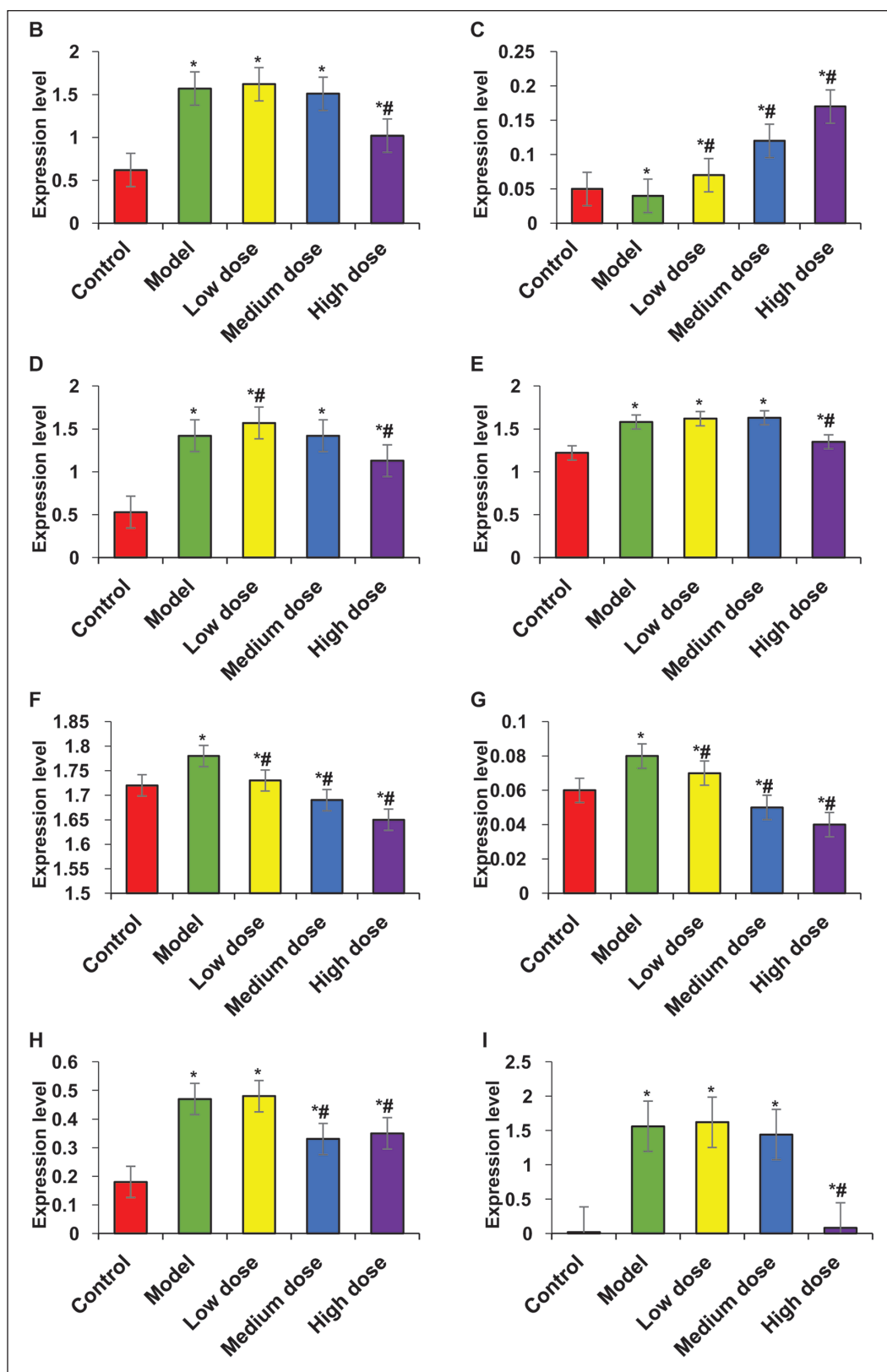


Figure 6. Influence of Raspberry Extract on Protein Levels of Endothelial Cells in Human Cerebral Aneurysms. (A) Western Blotting; (B) Bax; (C) Bcl-2; (D) Caspase8; (E) Cleaved-caspase8; (F) Caspase12; (G) Cleaved-caspase12; (H) Caspase3; (I) Cleaved-caspase3.

Note: * $p < 0.05$ versus C group; # $p < 0.05$ versus M group.

extract can inhibit endothelial cell apoptosis and vascular damage, improve vascular function, and prevent disease progression (Wang et al., 2021). Studying the components and mechanisms of action of raspberry extract helps uncover its specific roles in apoptosis regulation and cellular function modulation, providing a theoretical and practical basis for developing new drugs and treatment strategies.

The advantages of this study are as follows. Raspberry extract has been demonstrated to mediate the PI3K/AKT signaling pathway, a finding that offers new avenues for improving the survival rate of cerebral endothelial cells and inhibiting cell apoptosis. First, the study provides an in-depth analysis of the molecular mechanisms underlying the effects of raspberry extract, which contributes to a better understanding of its biological activity in protecting cerebral endothelial cells. Second, the PI3K/AKT signaling pathway plays a crucial role in various biological processes, including cell survival, proliferation, and apoptosis. The results of this study expand the potential applications of this pathway in the treatment of cerebrovascular diseases. Moreover, as a natural plant-derived compound, raspberry extract may offer superior biocompatibility and fewer side effects, providing strong support for the development of novel therapeutic agents for cerebrovascular diseases in the future. Nevertheless, the study has certain limitations: Although raspberry extract has shown positive effects in mediating the PI3K/AKT signaling pathway, there are still several limitations. First, the experimental subjects were limited to human cerebral endothelial cells, with no inclusion of animal models or clinical trials, so the applicability of the results requires further validation. Second, the regulatory mechanisms of the PI3K/AKT pathway within cells are complex, and the precise molecular targets of raspberry extract within this pathway require further investigation. Additionally, the study did not explore the potential interactions between raspberry extract and other drugs, and the safety and efficacy of the extract in practical applications need to be confirmed through further research. Therefore, future studies should take these factors into account and aim to refine and expand the research findings in this field.

Conclusion

Raspberry extract can enhance the survival rate of cerebral VECs, with a more pronounced effect observed, especially in the HD group. This may be attributed to the protective effects of the components in raspberry extract, which bolster cell survival by shielding them from damage. Raspberry extract can reduce the apoptotic levels of cerebral VECs, particularly with a more pronounced modulation effect seen in the HD group. Specifically, the HD group of raspberry extract can decrease levels of pro-apoptotic factors while increasing the expression of anti-apoptotic factors like Bcl-2. Moreover, raspberry extract can also reduce inflammatory factors in cerebral VECs, including MMP-9, IL-17A, and ET-1.

Raspberry extract can elevate the levels of PI3K and AKT in cerebral VECs. PI3K/AKT signaling is a crucial cell survival pathway, and its activation can promote cell survival while inhibiting apoptosis. Raspberry extract exhibits a protective effect on cerebral VECs, potentially contributing to the reduction in the occurrence and progression of cerebral aneurysms. Nevertheless, further research is required to validate and clarify its detailed molecular mechanisms.

Abbreviations

BCA: Bicinchoninic acid; DMSO: Dimethyl sulfoxide; ECL: Enhanced chemiluminescence; ET: Endothelin; HD: High-dose; HPLC: High-performance liquid chromatography; IL: Interleukin; LD: Low-dose; M: Model; MD: Medium-dose; MMP-9: Matrix metalloproteinase-9; MTT: 3-(4,5)-Dimethylthiazolyl-2,5-diphenyltetrazolium bromide; TBST: Tris-hydrochloric acid buffer solution; VECs: Vascular endothelial cells.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

None.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Shiwen Guo  <https://orcid.org/0009-0008-5312-4409>

References

- Brie, D. A., Jianu, A. M., Popescu, R., Marcovici, T. M., Muntean, I., Puscasiu, D., Mituletu, M., Brie, D. M., & Boruga, M. (2024). A review of abdominal aortic aneurysms: Risk factors, screening, pharmacological approach. *Farmacacia*, 72(2), 262–272. <https://doi.org/10.31925/farmacacia.2024.2.3>
- Chen, Y., Chen, Z., Guo, Q., Gao, X., Ma, Q., Xue, Z., Ferri, N., Zhang, M., & Chen, H. (2019). Identification of ellagitannins in the unripe fruit of *Rubus chingii* Hu and evaluation of its potential antidiabetic activity. *Journal of Agricultural and Food Chemistry*, 67(25), 7025–7039. <https://doi.org/10.1021/acs.jafc.9b02293>
- Chen, Z., Jiang, J., Shu, L., Li, X., Huang, J., Qian, B., Wang, X., Li, X., Chen, J., & Xu, H. (2021). Combined transcriptomic and metabolic analyses reveal potential mechanism for fruit development and quality control of Chinese raspberry (*Rubus chingii* Hu). *Plant Cell Reports*, 40(10), 1923–1946. <https://doi.org/10.1007/s00299-021-02758-6>
- Chen, Y., Xu, L., Wang, Y., Chen, Z., Zhang, M., & Chen, H. (2020). Characterization and functional properties of a pectin/tara gum based edible film with ellagitannins from the unripe fruits of *Rubus chingii* Hu. *Food Chemistry*, 325, 126964. <https://doi.org/10.1016/j.foodchem.2020.126964>

- Chou, W. H., Oinaka, T., Kanamaru, F., Mizutani, K., Chen, F. H., & Tanaka, O. (1987). Diterpene glycosides from leaves of Chinese *Rubus chingii* and fruits of *R. suavissimus*, and identification of the source plant of the Chinese folk medicine “fu-pen-zi”. *Chemical and Pharmaceutical Bulletin*, 35(7), 3021–3024. <https://doi.org/10.1248/cpb.35.3021>
- Cyboran-Mikołajczyk, S., Męczarska, K., Solarska-Ściuk, K., Ratajczak-Wielgomas, K., Oszmiański, J., Jencova, V., & Bonarska-Kujawa, D. (2022). Protection of erythrocytes and microvascular endothelial cells against oxidative damage by *Fragaria vesca* L. and *Rubus idaeus* L. leaves extracts-The mechanism of action. *Molecules* (Basel, Switzerland), 27(18), 5865. <https://doi.org/10.3390/molecules27185865>
- Ding, H.-Y. (2011). Extracts and constituents of *Rubus chingii* with 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. *International Journal of Molecular Sciences*, 12(6), 3941–3949. <https://doi.org/10.3390/ijms12063941>
- Han, B., Chen, J., Yu, Y. Q., Cao, Y. B., & Jiang, Y. Y. (2016). Antifungal activity of *Rubus chingii* extract combined with fluconazole against fluconazole-resistant *Candida albicans*. *Microbiology and Immunology*, 60(2), 82–92. <https://doi.org/10.1111/1348-0421.12357>
- He, Y., Jin, S., Ma, Z., Zhao, J., Yang, Q., Zhang, Q., Zhao, Y., & Yao, B. (2020). The antioxidant compounds isolated from the fruits of Chinese wild raspberry *Rubus chingii* Hu. *Natural Product Research*, 34(6), 872–875. <https://doi.org/10.1080/14786419.2018.1504046>
- Hsieh, T.-C., Lu, X., Guo, J., Xiong, W., Kunicki, J., Darzynkiewicz, Z., & Wu, J. M. (2002). Effects of herbal preparation Equiguard on hormone-responsive and hormone-refractory prostate carcinoma cells: Mechanistic studies. *International Journal of Oncology*, 20(4), 681–689. <https://doi.org/10.3892/ijo.20.4.681>
- Hua, Y.-J., Xie, F., Mao, K.-J., Luo, Y.-Y., & Ding, Y.-J. (2023). Insights into the metabolite profiles of *Rubus chingii* Hu at different developmental stages of fruit. *Journal of Separation Science*, 46(16), e2300264. <https://doi.org/10.1002/jssc.202300264>
- Jiang, M.-J., Li, L., Huang, W.-F., Su, J., Li, Y.-H., Qu, X.-S., & Fan, L.-L. (2021). *Rubus chingii* var. *suavissimus* alleviates high-fat diet-induced lipid metabolism disorder via modulation of the PPARs/SREBP pathway in Syrian golden hamsters. *Journal of Natural Medicines*, 75(4), 884–892. <https://doi.org/10.1007/s11418-021-01536-8>
- Jin, H. P., Cai, K. R., Yuan, C. H., & Yang, X. Q. (2023). *Acorus tatarinowii* Schott increases the permeability of blood-retinal barrier: Evidence from an *in vitro* study. *Farmacia*, 71(1), 205–209. <https://doi.org/10.31925/farmacia.2023.1.23>
- Ke, H., Bao, T., & Chen, W. (2019). Polysaccharide from *Rubus chingii* Hu affords protection against palmitic acid-induced lipotoxicity in human hepatocytes. *International Journal of Biological Macromolecules*, 133, 1063–1071. <https://doi.org/10.1016/j.ijbiomac.2019.04.176>
- Ke, H., Bao, T., & Chen, W. (2021). New function of polysaccharide from *Rubus chingii* Hu: Protective effect against ethyl carbamate induced cytotoxicity. *Journal of the Science of Food and Agriculture*, 101(8), 3156–3164. <https://doi.org/10.1002/jsfa.10944>
- Kong, Y., Hu, Y., Li, J., Cai, J., Qiu, Y., & Dong, C. (2022). Anti-inflammatory effect of a novel pectin polysaccharide from *Rubus chingii* Hu on colitis mice. *Frontiers in Nutrition*, 9, 868657. <https://doi.org/10.3389/fnut.2022.868657>
- Lei, T., Huang, J., Ruan, H., Qian, W., Fang, Z., Gu, C., Zhang, N., Liang, Y., Wang, Z., Gao, L., & Wang, Y. (2023). Competition between FLS and DFR regulates the distribution of flavonols and proanthocyanidins in *Rubus chingii* Hu. *Frontiers in Plant Science*, 14, 1134993. <https://doi.org/10.3389/fpls.2023.1134993>
- Li, H., Li, Y., Zhang, Y., Tong, L., Sa, Y., & Sun, W. (2023). *Rubus chingii* Hu relieved the polycystic ovary syndrome with enhanced insulin sensitivity through inhibiting TXNIP/NLRP3 inflammasome signaling. *Gynecological Endocrinology*, 39(1), 2237116. <https://doi.org/10.1080/09513590.2023.2237116>
- Li, X., Wang, Y., Jin, L., Chen, Z., Jiang, J., & Jackson, A. (2021). Development of fruit color in *Rubus chingii* Hu (Chinese raspberry): A story about novel offshoots of anthocyanin and carotenoid biosynthesis. *Plant Science*, 311, 110996. <https://doi.org/10.1016/j.plantsci.2021.110996>
- Luo, H., Ying, N., Zhao, Q., Chen, J., Xu, H., Jiang, W., Wu, Y., Wu, Y., Gao, H., & Zheng, H. (2023). A novel polysaccharide from *Rubus chingii* Hu unripe fruits: Extraction optimization, structural characterization and amelioration of colonic inflammation and oxidative stress. *Food Chemistry*, 421, 136152. <https://doi.org/10.1016/j.foodchem.2023.136152>
- Wan, J., Wang, X.-J., Guo, N., Wu, X.-Y., Xiong, J., Zang, Y., Jiang, C.-X., Han, B., Li, J., & Hu, J.-F. (2021). Highly oxygenated triterpenoids and diterpenoids from fructus Rubi (*Rubus chingii* Hu) and their NF-kappa B inhibitory effects. *Molecules*, 26(7), 1911. <https://doi.org/10.3390/molecules26071911>
- Wang, Q., Huang, Z., Gao, C., Ge, Y., & Cheng, R. (2021). The complete chloroplast genome sequence of *Rubus hirsutus* Thunb. and a comparative analysis within *Rubus* species. *Genetica*, 149 (5–6), 299–311. <https://doi.org/10.1007/s10709-021-00131-9>
- Wang, Y., Fang, M., Ren, Q., Qi, W., Bai, X., Amin, N., Zhang, X., Li, Z., & Zhang, L. (2024). Sox17 protects human brain microvascular endothelial cells from AngII-induced injury by regulating autophagy and apoptosis. *Molecular and Cellular Biochemistry*, 479(9), 2337–2350. <https://doi.org/10.1007/s11010-023-04838-5>
- Wang, J., Xiao, X., Zhou, N., Zhao, M., Lang, S., Ren, Q., Wang, D., & Fu, H. (2023). Rubochingosides A–J, labdane-type diterpene glycosides from leaves of *Rubus chingii*. *Phytochemistry*, 210, 113670. <https://doi.org/10.1016/j.phytochem.2023.113670>
- Wu, J., Zhang, D., Zhu, B., Wang, S., Xu, Y., Zhang, C., Yang, H., Wang, S., Liu, P., Qin, L., & Liu, W. (2022). *Rubus chingii* Hu. unripe fruits extract ameliorates carbon tetrachloride-induced liver fibrosis and improves the associated gut microbiota imbalance. *Chinese Medicine*, 17(1), 56. <https://doi.org/10.1186/s13020-022-00607-6>
- Xu, T., Chen, H., Chen, H., Li, K., Wang, Y., Dou, M., & Chen, J. (2023). First report of black patch disease caused by *Phyllosticta capitalensis* on *Litsea cubeba* in China. *Plant Disease*, 107(9), 2883. <https://doi.org/10.1094/PDIS-12-22-2792-PDN>
- Xu, W., Zhao, M., Fu, X., Hou, J., Wang, Y., Shi, F., & Hu, S. (2021). Molecular mechanisms underlying macrophage immunomodulatory activity of *Rubus chingii* Hu polysaccharides. *International Journal of Biological Macromolecules*, 185, 907–916. <https://doi.org/10.1016/j.ijbiomac.2021.07.024>
- Yang, Y., Zhang, K., Xiao, Y., Zhang, L., Huang, Y., Li, X., Chen, S., Peng, Y., Yang, S., Liu, Y., & Cheng, F. (2022). Genome assembly and population resequencing reveal the geographical divergence of Shanmei (*Rubus corchorifolius*). *Genomics Proteomics*

- Bioinformatics*, 20(6), 1106–1118. <https://doi.org/10.1016/j.gpb.2022.05.003>
- Yau, M.-H., Che, C.-T., Liang, S.-M., Kong, Y.-C., & Fong, W.-P. (2002). An aqueous extract of *Rubus chingii* fruits protects primary rat hepatocytes against tert-butyl hydroperoxide induced oxidative stress. *Life Sciences*, 72(3), 329–338. [https://doi.org/10.1016/s0024-3205\(02\)02239-7](https://doi.org/10.1016/s0024-3205(02)02239-7)
- Zan, T., Piao, L., Wei, Y., Gu, Y., Liu, B., & Jiang, D. (2018). Simultaneous determination and pharmacokinetic study of three flavonoid glycosides in rat plasma by LC-MS/MS after oral administration of *Rubus chingii* Hu extract. *Biomedical Chromatography*, 32(3), e4106. <https://doi.org/10.1002/bmc.4106>
- Zeng, H.-J., Liu, Z., Wang, Y.-P., Yang, D., Yang, R., & Qu, L.-B. (2018). Studies on the anti-aging activity of a glycoprotein isolated from Fupenzi (*Rubus chingii* Hu.) and its regulation on klotho gene expression in mice kidney. *International Journal of Biological Macromolecules*, 119, 470–476. <https://doi.org/10.1016/j.ijbiomac.2018.07.157>
- Zhang, X.-Y., Li, W., Wang, J., Li, N., Cheng, M.-S., & Koike, K. (2019). Protein tyrosine phosphatase 1B inhibitory activities of ursane-type triterpenes from Chinese raspberry, fruits of *Rubus chingii*. *Chinese Journal of Natural Medicines*, 17(1), 15–21. [https://doi.org/10.1016/S1875-5364\(19\)30004-4](https://doi.org/10.1016/S1875-5364(19)30004-4)
- Zhang, T. T., Liu, Y.-J., Yang, L., Jiang, J.-G., Zhao, J.-W., & Zhu, W. (2017). Extraction of antioxidant and antiproliferative ingredients from fruits of *Rubus chingii* Hu by active tracking guidance. *MedChemComm*, 8(8), 1673–1680. <https://doi.org/10.1039/c7md00240h>
- Zhang, W., Su, D., & Sun, R. (2022). First report of *Phyllosticta capitalensis* causing black freckle disease on *Rubus chingii* in China. *Plant Disease*, 106(5), 1517. <https://doi.org/10.1094/PDIS-05-21-1031-PDN>
- Zhang, T.-T., Yang, L., & Jiang, J.-G. (2015). Bioactive comparison of main components from unripe fruits of *Rubus chingii* Hu and identification of the effective component. *Food and Function*, 6(7), 2205–2214. <https://doi.org/10.1039/c5fo00406c>
- Zhong, R., Guo, Q., Zhou, G., Fu, H., & Wan, K. (2015). Three new labdane-type diterpene glycosides from fruits of *Rubus chingii* and their cytotoxic activities against five tumor cell lines. *Fitoterapia*, 102, 23–26. <https://doi.org/10.1016/j.fitote.2015.01.007>
- Zhong, J., Wang, Y., Li, C., Yu, Q., Xie, J., Dong, R., Xie, Y., Li, B., Tian, J., & Chen, Y. (2022). Natural variation on free, esterified, glycosylated and insoluble-bound phenolics of *Rubus chingii* Hu: Correlation between phenolic constituents and antioxidant activities. *Food Research International*, 162(A), 112043. <https://doi.org/10.1016/j.foodres.2022.112043>