

Neuroprotective effects of hesperidin on cerebral vasospasm after subarachnoid hemorrhage in rats: an experimental study

Emrah Keskin,¹ Bayram Yılmaz,² Mehmet Selim Gel³

¹Department of Neurosurgery, Zonguldak Bulent Ecevit University Faculty of Medicine, Zonguldak-Türkiye

²Department of Pathology, Hitit University Faculty of Medicine, Çorum-Türkiye

³Department of Neurosurgery, Trabzon Kanuni Training and Research Hospital, Trabzon-Türkiye

ABSTRACT

BACKGROUND: Subarachnoid hemorrhage (SAH) is a severe neurological emergency with high morbidity and mortality, primarily due to vasospasm and delayed ischemia. Hesperidin (HSP), a natural flavonoid, possesses strong antioxidant and vasoprotective properties. This experimental animal study examined HSP's neuroprotective role in oxidative stress and vascular remodeling after SAH, focusing on the extracellular regulated kinase 5–Kruppel-like factor 2–endothelial nitric oxide synthase (Erk5-KLF2-eNOS) pathway.

METHODS: The study was conducted from 2021 to 2022. Forty female Wistar albino rats were divided into five groups: control (G1, n=8), sham (G2, n=8), SAH + Vehicle (G3, n=8), SAH with low-dose HSP (G4, n=8), and SAH with high-dose HSP (G5, n=8). SAH was induced using a double injection of homologous blood into the cisterna magna. Biochemical markers (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx], nitric oxide [NOS]), basilar artery morphometry, and molecular expressions (Erk5, p-Erk5, KLF2, eNOS) were evaluated 48 hours post-SAH.

RESULTS: SAH significantly increased oxidative stress and reduced vascular lumen diameter in untreated rats (G3). Both HSP-treated groups (G4 and G5) showed improved antioxidant enzyme levels (SOD, CAT, GPx) and near-normal NOS levels. Morphometric analysis demonstrated significant preservation of basilar artery lumen diameter in treated groups, with no significant changes in wall thickness. Molecular analysis revealed upregulation of the Erk5-KLF2-eNOS pathway, suggesting a role in vasodilation and mitigation of oxidative stress.

CONCLUSION: HSP protects against SAH-induced vasospasm and oxidative damage by enhancing antioxidant capacity and modulating the Erk5-KLF2-eNOS pathway, suggesting its therapeutic potential.

Keywords: Hesperidin; subarachnoid hemorrhage; rat; vasospasm.

INTRODUCTION

The incidence of subarachnoid hemorrhage (SAH) in the United States of America (USA) is between 10 and 14 per 100,000 population per year, most commonly secondary to intracranial aneurysm rupture (6.9 to 9 per 100,000 in the USA).^[1-3] It is also a disease with high morbidity and mortality rates and can occur due to head trauma, arteriovenous malformation, or

bleeding diathesis without an underlying vascular pathology.^[4] Despite the development of new endovascular treatment methods in recent years (such as flow converters and intrasaccular devices) compared to the traditional microsurgical method, and despite the increased treatment of non-bleeding aneurysms, there has been no significant change in the morbidity and mortality rates of SAH.^[2,5]

Cite this article as: Keskin E, Yılmaz B, Gel MS. Neuroprotective effects of hesperidin on cerebral vasospasm after subarachnoid hemorrhage in rats: An experimental study. *Ulus Travma Acil Cerrahi Derg* 2025;31:1157-1167.

Address for correspondence: Emrah Keskin

Department of Neurosurgery, Zonguldak Bulent Ecevit University Faculty of Medicine, Zonguldak, Türkiye

E-mail: drkeskinemrah@gmail.com

Ulus Travma Acil Cerrahi Derg 2025;31(12):1157-1167 DOI: 10.14744/tjtes.2025.52931

Submitted: 12.10.2025 Revised: 17.10.2025 Accepted: 10.11.2025 Published: 16.12.2025

OPEN ACCESS This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).



The mortality rate, which is 15% at the time of aneurysm rupture, increases up to threefold in the following one-month period.^[2,6] The quality of life of survivors is reduced due to severe sequelae and neuropsychiatric disorders (such as depression and anxiety).^[7-9] All these negative outcomes are thought to be mainly due to late cerebral ischemia (secondary cerebral ischemia; SCI) following aneurysmal SAH.^[9] Despite the successful results obtained in many experimental studies on the treatment and prevention of vasospasm, which has been accepted as a precursor to the development of SCI for decades, there is still no clinical trial with acceptable results.^[10,11] The period characterized by autophagy, apoptosis, inflammation, destruction of the blood-brain barrier, global cerebral edema, and oxidative stress mechanisms and related factors that begins after aneurysm rupture is called early brain injury (24-72 hours).^[9,12] There is an urgent need for ongoing scientific research into new approaches for the prevention and treatment of early brain injury (EBI), which represents this period of pathophysiological destruction triggered by vasospasm.

Vasospasm, microthromboembolism, and neuronal damage, which are secondary complications of SAH, are closely related to oxidative stress. It has been previously shown in an experimental model of SAH that endothelial nitric oxide synthase (eNOS) uncoupling exacerbates oxidative stress and consequently secondary complications.^[13,14] Recently, the results of activation of the extracellular regulated kinase 5 (Erk5) pathway, modulated by eNOS and its activator, the transcription factor Kruppel-like factor 2 (KLF2), in attenuating vasospasm have been notable.^[15]

Hesperidin (HSP) is a bioactive flavonoid polymer abundantly present in citrus peels. HSP can directly cross the blood-brain barrier and alleviate hypoxia-induced blood-brain barrier dysfunction through its antioxidant action.^[16] In addition, it has exhibited antioxidative properties against spinal cord injury, subarachnoid hemorrhage, and cancer.^[17-19] Despite a previous study showing that HSP attenuates acute vasospasm after SAH, its mechanism of action is unclear.^[18] Therefore, this study evaluates the vasoprotective role of hesperidin via the Erk5-KLF2-eNOS pathway and highlights its potential as a natural flavonoid polymer in cerebrovascular therapy.

MATERIALS AND METHODS

Animals

This experimental study was approved by the Ethics Committee of Zonguldak Bülent Ecevit University and was conducted in the Research and Animal Laboratory of the same university (02-28-2021/01). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

The study was carried out between March 2021 and February 2022. Forty female Wistar albino rats weighing 350-400

g each were included in the study. All animals were housed at room temperature (22-25°C) under a diurnal (12:12-hour day/night) cycle with free access to food and water throughout the experiment.

Groups

Forty female Wistar albino rats (300-350 g) were divided into the following groups: Control group (G1, n=8, not subjected to any treatment or intervention); Sham + vehicle group (G2, n=8, subjected to sham experimental intervention and administered subarachnoid physiological saline); SAH + vehicle group (G3, n=8, subjected to SAH and administered subarachnoid physiological saline); SAH + low-dose HSP (G4, n=8, subjected to SAH and administered 100 mg/kg HSP, Sigma-Aldrich, USA); and SAH + high-dose HSP (G5, n=8, subjected to SAH and administered 300 mg/kg HSP, Sigma-Aldrich, USA). Subarachnoid hemorrhage was induced by a double injection of homologous blood into the cisterna magna as described by Pedard et al.^[20] The second SAH model was performed 24 hours after the first.

Study Design (Induction of SAH by the Double-Injection Homologous Blood Method in Rats)

All rats were anesthetized with ketamine (4 mg/100 g) and xylazine (1.5 mg/100 g) intraperitoneally, and the hair was shaved from the suboccipital region to the neck. After the rats were placed in the prone position, their heads were tilted 30° forward and fixed. A midline skin incision was made between the occiput and atlas arch, and the muscles of the craniocervical region were dissected. The atlanto-occipital membrane was exposed. Hemostasis was achieved with electrocautery at all stages. A 26-gauge catheter filled with homologous arterial blood from the carotid artery of a rat of the same age and sex not included in the study was inserted through the vertical midline at a right angle to the atlanto-occipital membrane. To prevent an increase in intracranial pressure, 0.25 ml of cerebrospinal fluid was drawn into a syringe. Then, 0.25 mL of blood was injected into the cisterna magna in the first infusion. The second infusion was performed 24 hours after the first infusion, using the same surgical procedures. The second infusion was performed with the same amount of blood (0.25 mL) as in the first infusion. After both procedures, the rats were kept in an upside-down position (30°) on an inclined plane for 5 minutes. These two procedures were applied to all rats except the control group. The sham operation in Group 1 consisted of the same manipulation, but instead of blood, 0.25 mL of 0.09% NaCl (physiologic saline) was administered in the first procedure and 0.25 mL in the second procedure (24 hours later). Rats in G3 (100 mg/kg) and G4 (300 mg/kg) were administered HSP by oral gavage immediately after the first SAH procedure and every 12 hours for the following 48 hours. After all rats were euthanized with a high dose of anesthetic agent (pentobarbital, 200 mg/kg, Biovet, Ankara, Türkiye), samples were taken from the cerebral hemispheres (Fig. 1).

In addition, blood sampling from the tail artery was not performed due to our clinic's experience with tail blood collection. Percutaneous blood collection from the tail artery is difficult and prolongs surgical time. Open surgeries cause additional surgical stress. In this context, we preferred the homologous blood injection model described by Pedard et al.^[20]

Morphometric Method of Basilar Artery Analysis

Cerebellum specimens sent to pathology and kept in 10% buffered formaldehyde for 48 hours were cut transversely from the midline to visualize the basilar artery and then blocked. The blocked specimens were processed, and 5-micron serial sections were taken and stained with hematoxylin and eosin. The preparations were examined with a Nikon Eclipse Ni-U (Japan) microscope and evaluated by a single pathologist. Basilar arteries were visualized using the NIS-Elements D version 5.02 program. Images were taken at 200× magnification, and basilar artery diameters were measured at four points (3, 6, 9, and 12 o'clock) with the ImageJ version 1.2 program, and the thickest wall thickness was recorded.^[21]

Biochemical Analysis

Super Oxide Dismutase (SOD, U/ml)

The role of SOD is to accelerate the conversion of the toxic radical produced during oxidative energy processes

into hydrogen peroxide and molecular oxygen. This method uses xanthine and xanthine oxidase to produce superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride to form a red formazan dye. Superoxide dismutase activity is then measured by the degree of inhibition of this reaction (Relassay, Türkiye).

Catalase (CAT, U/ml)

This colorimetric assay consists of two steps. First, the sample is incubated with a known amount of hydrogen peroxide. The sample converts hydrogen peroxide into water and oxygen. This rate is proportional to the CAT concentration. The enzyme is then stopped, and after a fixed incubation time the remaining hydrogen peroxide is determined using a chromogen. The absorbance is measured at 405 nm and the results are expressed as U/ml (Relassay, Türkiye).

Glutathione Peroxidase (GPx, U/ml)

This method is based on the technique of Paglia and Valentine. Glutathione peroxidase (GPx) catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione, it is immediately converted back to the reduced form by simultaneous oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to nicotinamide adenine dinucleotide phosphate (NADP). The decrease in absorbance at 340 nm is measured (Relassay, Türkiye).

Nitric Oxide (NO, µmol/L)

Nitric oxide measurement was carried out based on the Griess method. Since NO has a very short half-life, it is rapidly converted to its metabolites nitrite and nitrate. Nitrite is measured directly, and nitrate is measured after its reduction to nitrite using Griess reagent. In the Griess method, the amino group of sulfanilamide reacts with nitrite in an acid medium, undergoes diazotization, and forms a purple azo product with naphthylethylenediamine (NED). After the resulting color is read at a wavelength of 540 nm in a spectrometer, plasma NO levels are calculated indirectly according to the calibration curve prepared using nitrite standards (Relassay, Türkiye).

Western Blot Analysis

Basilar arteries were isolated and harvested 48 hours after SAH. Membrane proteins were extracted using a protein extraction kit (RIPA, Merck, KGaA, Darmstadt, Germany) according to the manufacturer's instructions. Western blotting was performed as previously described, using the following primary antibodies: anti-eNOS (Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-KLF2 and anti-p-Erk5 (Santa Cruz Biotechnology, CA, USA); and anti-Erk5 (sc-398015, Santa Cruz Biotechnology, Santa Cruz, CA, USA) [22]. A chemiluminescence imaging system was used for imaging. Since this imaging system is based on the chemical luminol, a secondary antibody-specific substrate (enhanced chemiluminescence; ECL) was used. The membrane was incubated in an ECL solution prepared at a 1:1 ratio for 5 minutes in a dark

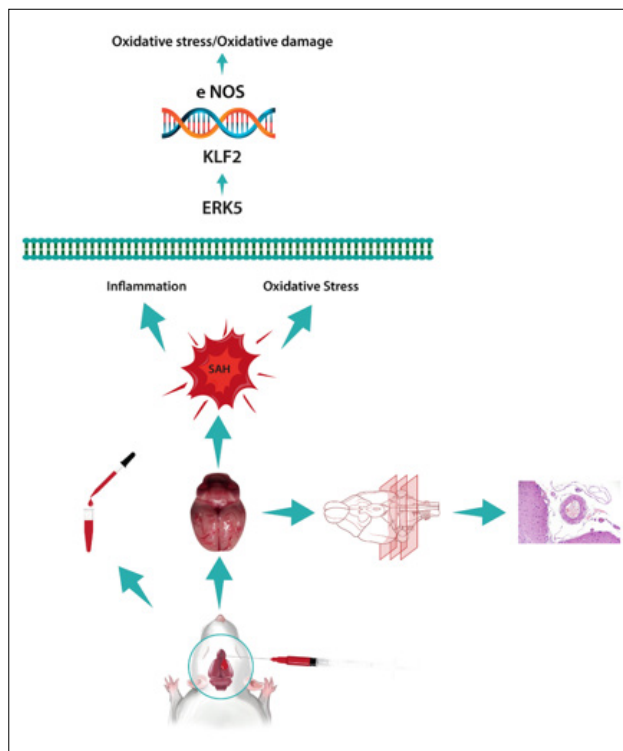


Figure 1. Schematic representation of the methodology used in this study. Blood and tissue samples were collected for histopathological and molecular analyses to assess oxidative stress markers and inflammatory responses. The diagram illustrates the experimental steps, including sample collection, oxidative stress assessment, and histological examination.

Table 1. The effects of hesperidin on superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and nitric oxide (NOS) levels in rat brain tissue following experimental subarachnoid hemorrhage

Groups/Parameters	n	SOD	CAT	GPX	NOS
G1	8	181.25±15.50	110.50±36.10	481.00±93.73	61.28±8.35
G2	8	147.00±12.72	98.25±22.17	396.25±103.68	55.22±8.37
G3	8	96.63±11.50	41.50±7.98	268.75±102.81	35.07±10.74
G4	8	131.75±9.54	83.13±13.29	424.13±164.01	51.17±13.30
G5	8	150.25±7.50	96.75±16.30	435.88±132.83	53.01±15.62
p*		55.697 (<0.001) ^{a,b,c,d,e,i,j}	12.407 (<0.001) ^{c,e,i,j}	3.438 (<0.05) ^b	5.641 (<0.01) ^{b,e}

Differences were evaluated by ANOVA and Kruskal-Wallis tests. Data are presented as mean±standard deviation. p* indicates significant differences at the 0.05 level. a: p<0.05 for G1 and G2 groups; b: p<0.05 for G1 and G3 groups; c: p<0.05 for G1 and G4 groups; d: p<0.05 for G1 and G5 groups; e: p<0.05 for G2 and G3 groups; f: p<0.05 for G2 and G4 groups; h: p<0.05 for G2 and G5 groups; i: p<0.05 for G3 and G4 groups; j: p<0.05 for G3 and G5 groups; k: p<0.05 for G4 and G5 groups.

environment, and imaging was initiated. Bands were detected with chemiluminescence imaging systems (G:BOX, Syngene, USA) and quantified according to band intensity using GeneSis software. β -Actin was used as an internal control.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences version 22.0. Descriptive statistics for quantitative variables were expressed as mean and standard deviation. The Shapiro-Wilk test was used to assess normal distribution assumptions. Skewness and kurtosis coefficients and dispersion in Q-Q plot graphs were also examined. Since the data were normally distributed for the parameters, the groups were compared using analysis of variance (ANOVA, San Diego, CA). Since the distribution was not normal for catalase, the relationship between the groups was analyzed using the Kruskal-Wallis test. When variances were assumed to be unequal, the Games-Howell test was used to determine statistical significance based on the mean scores of the groups. Results were considered statistically significant if the P value was <0.05.

RESULTS

Superoxide dismutase, CAT, GPx, and NOS values in all groups were analyzed 48 hours after the surgical procedure (Table 1). According to the SOD results, it was observed that G1 (control group) (181.25±15.50 U/ml) had the highest value; G2 (147.00±12.72 U/ml), G4 (131.75±9.54 U/ml), and G5 (150.25±7.50 U/ml) formed a cluster with similar effects; and the lowest value was observed in G3 (96.63±11.50 U/ml) (Table 1). The treatment groups (G4 and G5) showed a significant difference from the G1 (p=0.00<0.001) (Table 1) and G3 (p=0.00<0.001) (Table 1) groups, and no statistical difference was observed compared with the G2 group (p>0.05) (Table 1). Although SOD results were higher in G5 than in G4, no statistically significant difference was observed between these two groups (p>0.05) (Table 1). When the effect

of CAT measurement results on the groups was analyzed, the differences between G1 and G3 (p=0.01≤0.01), G2 and G3 (p=0.00<0.001), G4 and G3 (p=0.00<0.001), and G5 and G3 (p=0.00<0.001) were statistically significant (Table 1). When GPx results were analyzed, the highest value among the groups was observed in G1 (481.00±93.73 U/ml), followed by G5 (435.88±132.83 U/ml), G4 (424.13±164.01 U/ml), and G2 (396.25±103.68 U/ml), while the lowest value was observed in the G3 group (268.75±102.81 U/ml) (Table 1). A statistically significant difference between the groups was observed only between the G1 and G3 groups (p=0.03<0.05) (Table 1). When the NOS measurement results were analyzed, differences between the groups were observed between G1 and G2 (p=0.00<0.01) and between G2 and G3 (p=0.03<0.05) (Table 1).

Histopathologically, as expected, vessel samples from G1 showed a single layer of endothelium with elastic lamina and intima surrounded by smooth muscle cells, and the appearance of the intima was similar to that of the normal rat basilar artery (Fig. 2a). The arterial lumen was slightly dilated in the treatment groups (G4 and G5) compared to G1, G2, and G3. In G4 and G5, the epithelium appeared more intact, and flattening of the epithelium was observed compared to the other groups (Fig. 2b-c-d-e). While degenerative findings in the media layer increased in G2 and G3 compared to the other groups, the intima layer was also noted to be thicker in these groups compared to the other groups (Fig. 2b-c).

When the morphometric values of the basilar artery were evaluated, statistically significant differences were found between G4 and G5 compared to G3 (p=0.00<0.001), between G1 and G2 (p=0.03<0.05), and between G1 and G3 (p=0.00<0.01) (Table 2). However, no statistically significant difference was observed between G4 and G5 (p≥0.05) (Table 2). Regarding basilar artery wall thickness, no statistically significant differences were found, as similar results were observed in all groups (p>0.05) (Table 2).

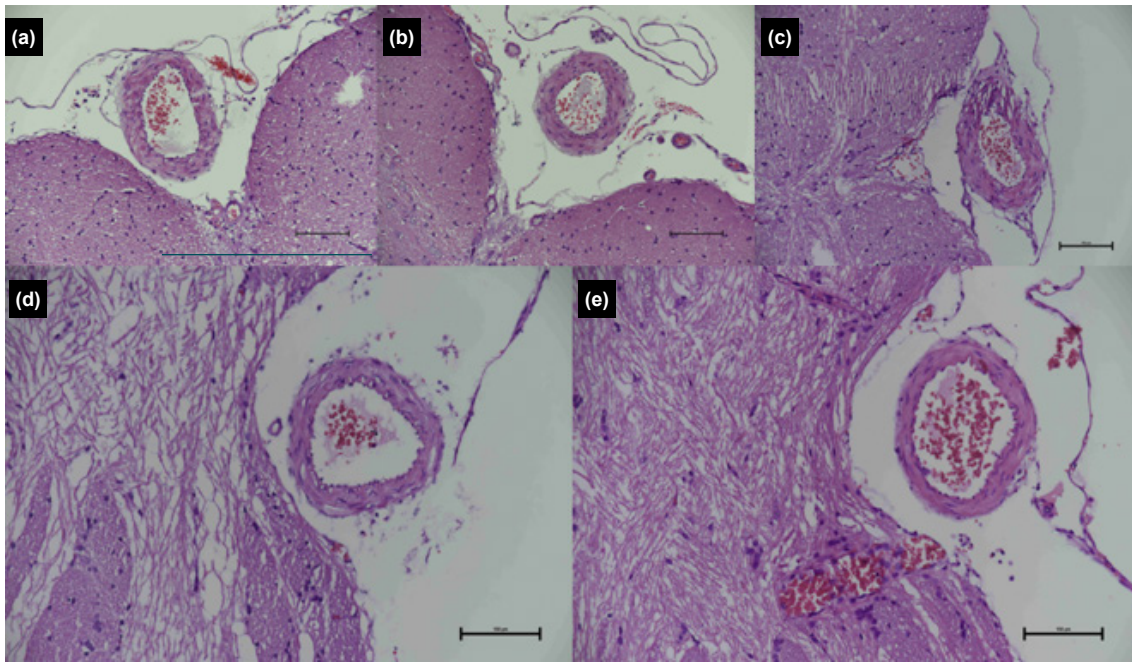


Figure 2. (a) Microscopic view of a normal basilar artery in Group 1 (hematoxylin and eosin, $\times 100$). (b) Microscopic view showing increased basilar artery wall thickness and luminal narrowing in Group 2 compared with Group 1 (hematoxylin and eosin, $\times 100$). (c) Microscopic view showing increased basilar artery wall thickness and luminal narrowing in Group 3 compared with Group 1 (hematoxylin and eosin, $\times 100$). (d) Microscopic view showing decreased basilar artery wall thickness and increased luminal narrowing in Group 4 compared with Group 2 (hematoxylin and eosin, $\times 100$). No statistically significant difference in basilar artery wall thickness was observed between Group 4 and Group 3, but basilar artery luminal diameters were markedly wider in Group 4. (e) Microscopic view showing decreased basilar artery wall thickness and increased luminal narrowing in Group 5 compared with Group 2 (hematoxylin and eosin, $\times 100$). No statistically significant difference in basilar artery wall thickness was observed between Group 5 and Group 3, but basilar artery luminal diameters were markedly wider in Group 5.

When the KLF2/ β -actin measurement results were analyzed, statistically significant differences were found between G1 and G2 ($p=0.00<0.001$), G3 ($p=0.00<0.001$), G4 ($p=0.00<0.001$), and G5 ($p=0.00<0.01$) (Fig. 3). Similarly, significant differences were observed between G2 and G4 ($p=0.00<0.001$) and G5 ($p=0.00<0.001$), and between G3 and G4 ($p=0.00<0.001$) and

G5 ($p=0.00<0.001$). However, the differences between G2 and G3 and between G4 and G5 were not statistically significant ($p>0.05$) (Table 3, Fig. 3).

ERK5/ β -actin measurement results were highest in G5 (0.91 ± 0.05) and lowest in G1 (0.50 ± 0.09). When the differ-

Table 2. Effect of hesperidin on rat basilar artery mean lumen diameter and wall thickness after experimental subarachnoid hemorrhage

Groups/Parameters	n	Wall Diameter	Wall Thickness
G1	8	121.63 \pm 24.49	39.46 \pm 7.03
G2	8	79.91 \pm 23.60	45.20 \pm 10.07
G3	8	69.98 \pm 7.69	47.76 \pm 5.24
G4	8	101.03 \pm 10.52	41.91 \pm 5.45
G5	8	107.32 \pm 12.60	41.07 \pm 6.42
p*		11.754 (<0.001) ^{a,b,l,j}	1.801 (>0.05)

Differences were evaluated by one-way ANOVA. Data are presented as mean \pm standard deviation. p* indicates a significant difference at the 0.05 level. a: $p<0.05$ for G1 and G2 groups; b: $p<0.05$ for G1 and G3 groups; c: $p<0.05$ for G1 and G4 groups; d: $p<0.05$ for G1 and G5 groups; e: $p<0.05$ for G2 and G3 groups; f: $p<0.05$ for G2 and G4 groups; h: $p<0.05$ for G2 and G5 groups; i: $p<0.05$ for G3 and G4 groups; j: $p<0.05$ for G3 and G5 groups; k: $p<0.05$ for G4 and G5 groups. No statistically significant differences were found between the groups with respect to wall thickness ($p>0.05$).

Table 3. Investigation of eNOS-β-actin, KLF2-β-actin, ERK5-β-actin, and p-ERK5-β-actin levels between groups

Group	n	eNOS-β-actin	KLF2-β-actin	ERK5-β-actin	pERK5-β-actin
G1	4	1.01±0.04	1.05±0.11	0.49±0.08	0.07±0.03
G2	4	0.88±0.09	0.31±0.04	0.59±0.10	0.11±0.02
G3	4	0.20±0.05	0.20±0.07	0.57±0.11	0.35±0.07
G4	4	0.77±0.07	0.73±0.04	0.74±0.08	0.76±0.04
G5	4	0.86±0.05	0.83±0.03	0.91±0.05	0.89±0.02
p		(<0.01) ^{b,c,e,h,i}	(<0.01) ^{a,b,c,d,f,g,h,i}	(<0.01) ^{c,d,g,i}	(<0.01) ^{b,c,d,e,f,g,h,i,j}

Statistical differences between groups are indicated by letters for p<0.01. Difference between Group 1 and Group 2 (a), difference between Group 1 and Group 3 (b), difference between Group 1 and Group 4 (c), difference between Group 1 and Group 5 (d), difference between Group 2 and Group 3 (e), difference between Group 2 and Group 4 (f), difference between Group 2 and Group 5 (g), difference between Group 3 and Group 4 (h), difference between Group 3 and Group 5 (i), difference between Group 4 and Group 5 (j).

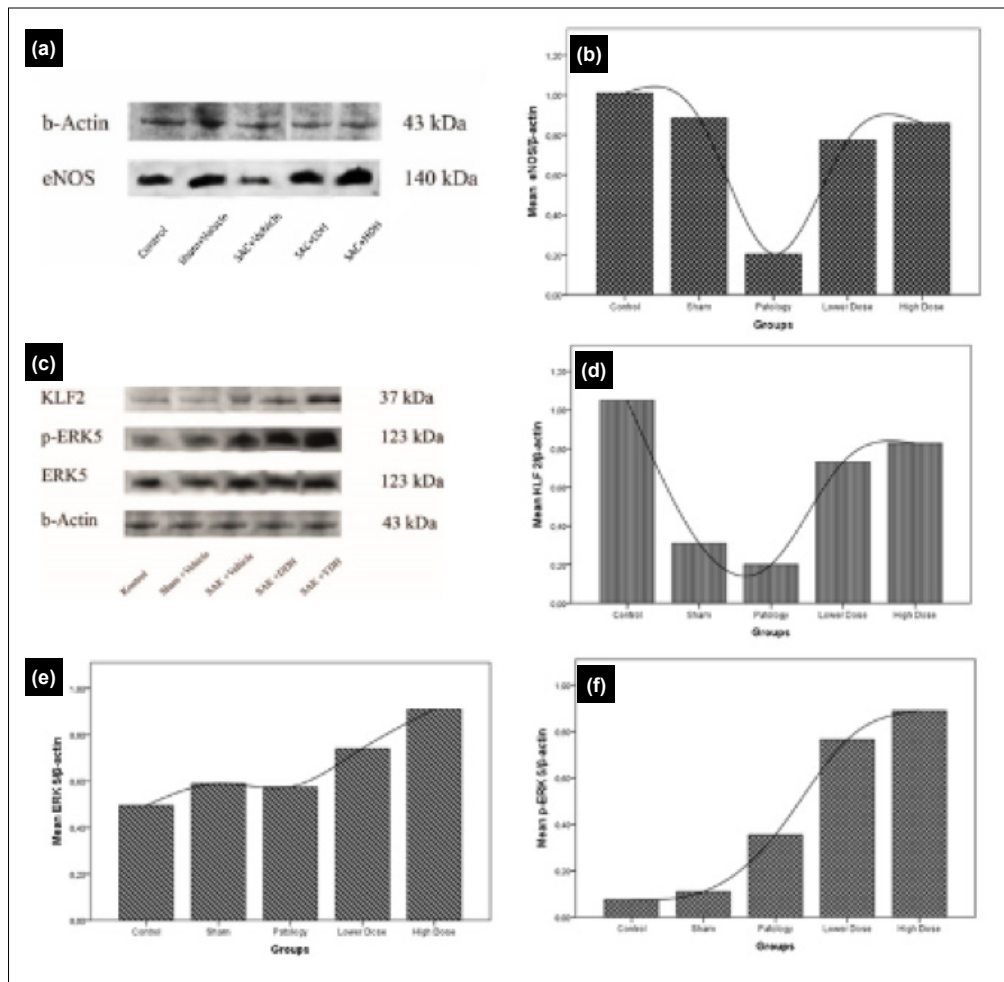


Figure 2. (a) Representative Western blot bands of eNOS expression in the hemisphere 48 hours after SAH. (b) Quantification analysis of eNOS expression in the hemisphere 48 hours after SAH (n=4). (c) Representative Western blot bands of Kruppel-like factor 2 (KLF2), phosphorylated-Erk5, and Erk5 expression. (d) Quantification analysis of KLF2 expression in the hemisphere 48 hours after SAH (n=4). (e) Quantification analysis of Erk5 expression in the hemisphere 48 hours after SAH (n=4). (f) Quantification analysis of p-Erk5 expression in the hemisphere 48 hours after SAH (n=4).

ences between the groups were analyzed, it was observed that the differences between G1 and G4 ($p=0.03<0.05$) and G5 ($p=0.00<0.001$), between G2 and G5 ($p=0.00<0.01$), and between G3 and G5 ($p=0.00<0.01$) were statistically significant (Table 3, Fig. 3).

When eNOS/ β -actin results were analyzed, the differences between G1 and G3 ($p=0.00<0.001$) and G4 ($p=0.00<0.01$), and between G3 and G2 ($p=0.00<0.001$), G4 ($p=0.00<0.001$), and G5 ($p=0.00<0.001$) were statistically significant (Table 3, Fig. 3).

When p-ERK5/ β -actin results were analyzed, it was found that only the difference between G1 and G2 was not statistically significant ($p>0.05$). Except for this comparison, it was observed that the differences between all other paired groups were statistically significant (Table 3, Fig. 3).

DISCUSSION

The first goal in the treatment of subarachnoid hemorrhage is to stop the bleeding and, if possible, address the underlying cause. Rebleeding is the most feared and most fatal complication, occurring in 40% of patients with a mortality rate of 80%. Even in cases where hemorrhage is completely controlled, guidelines recommend maintaining a mean arterial pressure above 110, systolic pressure in the range of 140-160, hemodilution, hypervolemia, normothermia, normoglycemia, normoglycemia, normonatremia, moderate hypercarbia (PaCO_2), and preventing seizures, hydrocephalus, and increased intracranial pressure through close monitoring.^[7,22-29]

Vasospasm is known to be the most common early-to-mid-term complication (days 3-14) of subarachnoid hemorrhage following rebleeding (40%). It is known that vasospasm, a condition that produces clinical findings through decreased cerebral perfusion leading to narrowing of the vessel lumen, causes delayed SCI in one-third of all subarachnoid hemorrhage cases.^[23,24,28-30] The development of SCI worsens prognosis by increasing mortality and morbidity 1.5- to 4-fold.^[28,31] Although there is no definitive cause of vasospasm, its pathophysiology is thought to be multifactorial and involves ischemia and immune destruction caused by blood elements crossing the blood-brain barrier, necrotic tissue damage, oxidative stress, and inflammation.^[23,32-36]

Many studies have shown that subarachnoid hemorrhage causes vasospasm through both signaling pathways stimulated by inflammation and the vasoconstrictive effects of irritant metabolites formed from necrotic neural tissues and accumulated blood products around the vessel wall on vascular smooth muscle.^[23,24,29,30,36-39] In addition to the above-mentioned measures taken to reduce risk factors, calcium-channel blockers and antagonists used to reduce vasospasm, which may be partially successful, can decrease the resulting vasoconstriction, but treatments capable of preventing vasospasm at the point where oxidant irritation begins have not yet been identified.^[28,39] Both the tissue destruction resulting

from ischemic tissue damage and the immune system elements involved in the inflammatory process caused by blood products (such as auto-oxidation of hemoglobin) increase oxidative stress in the tissue.^[40-42] Therefore, the total damage to the tissue is correlated with oxidative stress, and an agent such as HSP, whose antioxidant and anti-inflammatory effects have been demonstrated in the literature, is expected to reduce tissue damage and thus the delayed state of SCI due to vasospasm.^[34,35,43] This hypothesis is supported by literature demonstrating that treatments aimed at reducing the amount of blood in the subarachnoid space are successful in reducing vasospasm and GSI.^[37,44]

There are various experimental models available to simulate SCI due to vasospasm that develops after subarachnoid hemorrhage.^[20,45-47] Since the amount of blood in the subarachnoid space is among the parameters affecting the degree of vasospasm in this study, the model created by homologous blood injection into the cisterna magna, described by Pedard et al., a method in which the amount of blood in the subarachnoid space is known, was preferred.^[20] Due to the dense anastomoses in rat cerebral artery anatomy, it is impractical to demonstrate ischemia in micropathological examination because ischemia does not produce necrotic scars in the tissue.^[48] Therefore, the diameter of the basilar artery lumen examined in rapidly fixed specimens after decapitation was chosen as an indirect indicator of ischemia and a direct indicator of vasospasm.^[20] In addition, oxidative stress, which is a common result of inflammation and damage caused by subarachnoid hemorrhage, was closely evaluated using oxidant stress parameters measured by ELISA (enzyme-linked immunosorbent assay) and Western blot techniques.

Hesperidin is a molecule that has been extensively studied in the literature, and its anti-inflammatory, antioxidant, and neuroprotective properties have been repeatedly demonstrated in different models.^[49-55] Since this molecule is an antioxidant with neuroprotective effects, it appears suitable for use in SCI and vasospasm, where cerebral oxidant damage is prominent. Considering all these features, our study aimed to reveal the mechanism of the antioxidant effect of HSP, its relationship with the KLF2-ERK5-eNOS pathway, which is known to increase vasodilation and endothelial stability, and its effect on SCI.^[16-19,43]

In the literature, many studies indicate that subarachnoid hemorrhage increases oxidative stress and that there is a correlation between the quantitative level of this oxidative stress, the degree of vasospasm, and consequently the extent of SCI.^[34,35,40,42,44,56-60] Similar results were observed in our study. The number of oxidants in the environment decreases because of the activity of the SOD, CAT, and GPx enzymes, whose function is to neutralize free radicals. In this way, oxidants can be reduced, but the remaining oxidant capacity is also lowered. Therefore, the amount of decrease in these values is considered the portion of this capacity that is used in vivo and serves as an indicator of oxidative stress.^[34,35,42,56,57,60] While

all neutralizing capacity parameters were relatively higher in G1 than in the other groups, they decreased in all groups in which oxidant exposure was partially present. The lowest values were measured in the untreated SAH group (G3) for all parameters. Although statistically significant differences were not observed, particularly in the measurements of glutathione peroxidase, an enzyme that indirectly provides a neutralizing effect, and in some other measurements (Table 1), an antioxidant effect was noted in the treatment groups in terms of oxidant exposure, which brought the neutralizing capacity closer to the values of the non-SAH treatment group (G2). Although it could not be demonstrated that this effect increased dose-dependently with statistical significance, it can be said that the antioxidant efficacy of high-dose HSP was higher, albeit by a small margin.

Nitric oxide is an intracellular signaling molecule and neurotransmitter that is also thought to increase vascular tone, insulin release, angiogenesis, local smooth muscle reciprocal regulation, hypotension, and the shock state in septic immune responses. The production and concentration of NO are controlled by three main enzymes, of which eNOS and neuronal-derived NO synthetase predominate in the routine functioning of the body.^[61-63] However, during inflammatory processes, inducible NO synthetase (iNOS) becomes dominant, causing the oxidant character of NO to predominate. This promotes its use in the nonspecific immune response and enhances its vasodilatory effects, increasing the flow to the site and increasing the transport of inflammatory elements.^[64-66] Although this is part of a mechanism to protect the body, it also causes damage to parenchymal tissues and promotes the growth of inflammation by suddenly and substantially increasing oxidative stress in the region. When these damaged tissues include the vessel walls, a vasospasm response may occur.^[33,66-70] One of the active pathways that helps maintain eNOS dominance in NO production is the pathway initiated by the ERK5 receptor, which was examined in our study. The ERK5 receptor increases NO levels by upregulating eNOS production via the KLF2 transcription factor, in order to decrease intravascular flow velocity by stimulating endothelial cells in response to the entraining shear stress caused by the flow rate during intravascular flow. Since this increase in production occurs gradually over time, it allows adaptive mechanisms to develop to neutralize the oxidant stress caused by NO. In this way, while it does not create oxidant stress, it reduces shear stress through its vasodilating effect.^[15,71-73]

When the results of our study were analyzed, a significant statistical difference in NO production was found only between the untreated control group (G1) and the placebo treatment group without bleeding (G2), and the untreated SAH group (G3). The fact that the NO measurements in the treatment groups (G4 and G5) were not significantly different from those in the nonbleeding groups (G1 and G2) showed that HSP was able to increase NO levels to near-normal values (Table 1). When we combine this finding with the obser-

vation that oxidative stress was also reduced in the treatment groups, we conclude that HSP both supports NO production from more gradual sources rather than explosive iNOS-induced production in the treated groups, and prevents NO from cyclically aggravating oxidative stress and the inflammatory response, thus preventing NO depletion and creating a stabilizing effect.

When the expression levels of ERK5-KLF2-eNOS involved in NO production were examined (Fig. 3), the finding that ERK5 levels were significantly different in the G5 group compared to all other groups, and were higher in the two treatment groups than in all other groups without correlation with other parameters, led to the conclusion that HSP treatment caused ERK5 receptor upregulation, even though the process occurred in an acute-subacute phase. The p-ERK5/ERK5 ratio reflects the degree of ERK5 receptor activation, the first key step in the ERK5-KLF2-eNOS pathway.^[15] While ERK5 receptor activation was very low in the first two groups, a moderate level of activation was observed in G3. This partial increase in activation was interpreted as the effect of increased shear stress due to vasospasm. The higher ERK5 activation in both treatment groups compared to G3 suggests that one of the mechanisms by which HSP increases NO is through the KLF2-eNOS pathway. The parallel increase of eNOS and KLF2 expression and the significant differences in these values between the untreated SAH group (G3) and the treatment groups (G4 and G5) further suggest that activation of the eNOS pathway is enhanced by HSP and can lead to the end product, i.e., an increase in eNOS-derived NO, without encountering intracellular resistance. When these values are considered together, the ranking of ERK5 activation (with the treatment groups G4 and G5 followed by the untreated SAH group G3), contrasted with the intracellular conduction pathways (KLF2-eNOS) and the end product (NO) ranking last, supports both the idea that iNOS-induced NO production is dominant in G3 and that eNOS-induced production is somehow disrupted. It also supports the idea that NO is consumed by causing vascular and surrounding tissue irritation due to oxidative damage, rather than exerting a vasodilatory effect, since most of the NO produced in G3 is iNOS-derived and increases oxidative damage by rising suddenly. In light of this information, it was concluded that HSP exerts part of its protective vasodilatory and antioxidant effects against vasospasm via the ERK5-KLF2-eNOS pathway.

Since the results of morphometric evaluation are the most important findings that directly demonstrate vasospasm in vitro, this evaluation has the greatest impact on the conclusions. According to the results of the statistical analysis of lumen diameters (Table 2), the significant differences between the control group (G1), the bleeding-free procedure group (G2), and the untreated SAH group (G3) indicate that vasospasm was successfully induced in the experimental model. The fact that there was no statistically significant difference between the control group (G1) and the treatment groups

(G4 and G5), although the values were not exactly the same, shows that HSP was able to successfully prevent vasospasm in the treatment groups (G4 and G5), which were not statistically distinguishable from the control group (Table 2). The lack of a statistically significant difference in wall thickness is an expected result, since vascular remodeling seen in the chronic period has not yet begun in our study, which reflects the acute-subacute period.

CONCLUSION

The antioxidant effect of HSP in the treatment groups, the stabilizing effect that prevents NO depletion by reducing oxidant stress and limiting immune overreaction, and the vasodilatory effect caused by gradual NO production through the ERK5-KLF2-eNOS pathway, which brings the vessel lumen diameter almost to the level observed in tissues without subarachnoid hemorrhage, indicate that this natural flavonoid polymer may be a promising treatment option for vasospasm and late SCI that may develop after subarachnoid hemorrhage.

Acknowledgments: We would like to thank Cansu Başar for her contributions to statistical analysis, Baran Medikal for his contributions to biochemical analysis, and Enago for language editing.

Ethics Committee Approval: This study was approved by the Zonguldak Bülent Ecevit University Animal Experiments Ethics Committee (Date: 28.01.2021, Decision No: 2021/01).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: E.K., M.S.G.; Design: E.K.; Supervision: E.K., B.Y.; Resource: E.K., M.S.G.; Materials: E.K.; Data collection and/or processing: M.S.G.; Analysis and/or interpretation: B.Y.; Literature review: M.S.G.; Writing: E.K.; Critical review: E.K., M.S.G.

Conflict of Interest: None declared.

Financial Disclosure: This study was conducted with the financial support of Zonguldak Bülent Ecevit University Scientific Research Projects Coordinatorship (2021-59510135-01).

REFERENCES

- Muehlschlegel S. Subarachnoid Hemorrhage. *Continuum (Minneapolis)* 2018;24:1623–57. [\[CrossRef\]](#)
- Ertman N, Chang HS, Hackenberg K, de Rooij NK, Vergouwen MDI, Rinkel GJE, et al. Worldwide Incidence of Aneurysmal Subarachnoid Hemorrhage According to Region, Time Period, Blood Pressure, and Smoking Prevalence in the Population: A Systematic Review and Meta-analysis. *JAMA Neurol* 2019;76:588–97. [\[CrossRef\]](#)
- Ji C, Chen G. Signaling Pathway in Early Brain Injury after Subarachnoid Hemorrhage: News Update. *Acta Neurochir Suppl* 2016;121:123–6. [\[CrossRef\]](#)
- Zhang J, Yuan G, Liang T, Pan P, Li X, Li H et al. Nix Plays a Neuroprotective Role in Early Brain Injury After Experimental Subarachnoid Hemorrhage in Rats. *Front Neurosci* 2020;14:245. [\[CrossRef\]](#)
- Yilmaz U, Garner M, Reith W. Subarachnoidalblutung, Teil 2: Therapie, Komplikationen und Langzeitfolgen. *Radiologie (Heidelberg)* 2025;65:525–34. [\[CrossRef\]](#)
- Chan V, O’Kelly C. Response by Chan and O’Kelly to Letter Regarding Article, "Declining Admission and Mortality Rates for Subarachnoid Hemorrhage in Canada Between 2004 and 2015". *Stroke* 2019;50:e133. [\[CrossRef\]](#)
- Connolly ES Jr, Rabinstein AA, Carhuapoma JR, Derdeyn CP, Dion J, Higashida RT, et al; American Heart Association Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; Council on Cardiovascular Surgery and Anesthesia; Council on Clinical Cardiology. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2012;43:1711–37. [\[CrossRef\]](#)
- Eagles ME, Tso MK, Macdonald RL. Cognitive Impairment, Functional Outcome, and Delayed Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage. *World Neurosurg* 2019;124:e558–62. [\[CrossRef\]](#)
- Osgood ML. Aneurysmal Subarachnoid Hemorrhage: Review of the Pathophysiology and Management Strategies. *Curr Neurol Neurosci Rep* 2021;21:50. [\[CrossRef\]](#)
- Zhang XH, Peng L, Zhang J, Dong YP, Wang CJ, Liu C, et al. Berberine Ameliorates Subarachnoid Hemorrhage Injury via Induction of Sirtuin 1 and Inhibiting HMGB1/NF- κ B Pathway. *Front Pharmacol* 2020;11:1073. [\[CrossRef\]](#)
- Güleç İ, Şengelen A, Karagöz-Güzey F, Önay-Uçar E, Eren B, Vahabova G, et al. The calcimimetic R-568 attenuates subarachnoid hemorrhage-induced vasospasm through PI3K/Akt/eNOS signaling pathway in the rat model. *Brain Res* 2021;1765:147508. [\[CrossRef\]](#)
- Fujii M, Yan J, Rolland WB, Soejima Y, Caner B, Zhang JH. Early brain injury, an evolving frontier in subarachnoid hemorrhage research. *Transl Stroke Res* 2013;4:432–46. [\[CrossRef\]](#)
- Sabri M, Ai J, Knight B, Tariq A, Jeon H, Shang X, et al. Uncoupling of endothelial nitric oxide synthase after experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2011;31:190–9. [\[CrossRef\]](#)
- Sabri M, Ai J, Marsden PA, Macdonald RL. Simvastatin re-couples dysfunctional endothelial nitric oxide synthase in experimental subarachnoid hemorrhage. *PLoS One* 2011;6:e17062. [\[CrossRef\]](#)
- Li Q, Chen Y, Zhang X, Zuo S, Ge H, Chen Y, et al. Scutellarin attenuates vasospasm through the Erk5-KLF2-eNOS pathway after subarachnoid hemorrhage in rats. *J Clin Neurosci* 2016;34:264–70. [\[CrossRef\]](#)
- Lee BK, Hyun SW, Jung YS. Yuzu and Hesperidin Ameliorate Blood-Brain Barrier Disruption during Hypoxia via Antioxidant Activity. *Antioxidants (Basel)* 2020;9:843. [\[CrossRef\]](#)
- Heo SD, Kim J, Choi Y, Ekanayake P, Ahn M, Shin T. Hesperidin improves motor disability in rat spinal cord injury through anti-inflammatory and antioxidant mechanism via Nrf-2/HO-1 pathway. *Neurosci Lett* 2020;715:134619. [\[CrossRef\]](#)
- Aydogmus E, Gul S, Bahadır B. Neuroprotective Effects of Hesperidin on Cerebral Vasospasm After Experimental Subarachnoid Hemorrhage in Rats: Biochemical, Pathologic, and Histomorphometric Analysis. *World Neurosurg* 2019;122:e1332–7. [\[CrossRef\]](#)
- Ferreira de Oliveira JMP, Santos C, Fernandes E. Therapeutic potential of hesperidin and its aglycone hesperetin: Cell cycle regulation and apoptosis induction in cancer models. *Phytomedicine* 2020;73:152887. [\[CrossRef\]](#)
- Pedard M, El Amki M, Lefevre-Scelles A, Compère V, Castel H. Double Direct Injection of Blood into the Cisterna Magna as a Model of Subarachnoid Hemorrhage. *J Vis Exp* 2020;(162). [\[CrossRef\]](#)
- Yan J, Chen C, Lei J, Yang L, Wang K, Liu J, et al. 2-methoxyestradiol reduces cerebral vasospasm after experimental subarachnoid hemorrhage in rats. *Exp Neurol* 2006;202:348–56. [\[CrossRef\]](#)
- Zuo S, Li W, Li Q, Zhao H, Tang J, Chen Q, et al. Protective effects of Ephedra sinica extract on blood-brain barrier integrity and neurological function correlate with complement C3 reduction after subarachnoid hemorrhage in rats. *Neurosci Lett* 2015;609:216–22. [\[CrossRef\]](#)
- Thilak S, Brown P, Whitehouse T, Gautam N, Lawrence E, Ahmed Z, et al. Diagnosis and management of subarachnoid haemorrhage. *Nat Com-*

- mun 2024;15:1850. [CrossRef]
24. van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. *Lancet* 2007;369:306–18. [CrossRef]
 25. Thompson BG, Brown RD Jr, Amin-Hanjani S, Broderick JP, Cockroft KM, Connolly ES Jr, et al; American Heart Association Stroke Council; Council on Cardiovascular and Stroke Nursing, and Council on Epidemiology and Prevention; American Heart Association; American Stroke Association. Guidelines for the Management of Patients With Unruptured Intracranial Aneurysms. *Stroke* 2015;46:2368–400. [CrossRef]
 26. Vergouwen MD, Vermeulen M, van Gijn J, Rinkel GJ, Wijdicks EF, Muijselaar JP, et al. Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. *Stroke* 2010;41:2391–5. [CrossRef]
 27. Galea JP, Dulhanty L, Patel HC; UK and Ireland Subarachnoid Hemorrhage Database Collaborators. Predictors of outcome in aneurysmal subarachnoid hemorrhage patients: observations from a multicenter data set. *Stroke* 2017;48:2958–63. [CrossRef]
 28. Dorhout Mees SM, Rinkel GJ, Feigin VL, Algra A, van den Bergh WM, Vermeulen M, et al. Calcium antagonists for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev* 2007;3:CD000277. [CrossRef]
 29. Claassen J, Park S. Spontaneous subarachnoid haemorrhage. *Lancet* 2022;400:846–62. [CrossRef]
 30. Brilstra EH, Rinkel GJ, Algra A, van Gijn J. Rebleeding, secondary ischemia, and timing of operation in patients with subarachnoid hemorrhage. *Neurology* 2000;55:1656–60. [CrossRef]
 31. Treggiari-Venzi MM, Suter PM, Romand JA. Review of medical prevention of vasospasm after aneurysmal subarachnoid hemorrhage: a problem of neurointensive care. *Neurosurgery* 2001;49:249–61. [CrossRef]
 32. Romoli M, Giannello F, Mosconi MG, De Mase A, De Marco G, Di Giovanni A, et al; IAY (Italian Stroke Association—Young Section). Immunological profile of vasospasm after subarachnoid hemorrhage. *Int J Mol Sci* 2023;24:8856. [CrossRef]
 33. Eisenhut M. Vasospasm in cerebral inflammation. *Int J Inflamm* 2014;2014:509707. [CrossRef]
 34. Shishido T, Suzuki R, Qian L, Hirakawa K. The role of superoxide anions in the pathogenesis of cerebral vasospasm. *Stroke* 1994;25:864–8. [CrossRef]
 35. Pyne-Geithman GJ, Caudell DN, Prakash P, Clark JF. Glutathione peroxidase and subarachnoid hemorrhage: implications for the role of oxidative stress in cerebral vasospasm. *Neurol Res* 2009;31:195–9. [CrossRef]
 36. Morooka H. Cerebral arterial spasm. II. Etiology and treatment of experimental cerebral vasospasm. *Acta Med Okayama* 1978;33:39–49.
 37. Spears WE, Greer DM, Nguyen TN. Comment on the 2023 guidelines for the management of patients with aneurysmal subarachnoid hemorrhage. *Stroke* 2023;54:2708–12. [CrossRef]
 38. Sakaki S, Kuwabara H, Ohta S. Biological defence mechanism in the pathogenesis of prolonged cerebral vasospasm in the patients with ruptured intracranial aneurysms. *Stroke* 1986;17:196–202. [CrossRef]
 39. Shah VA, Gonzalez LE, Suarez JJ. Therapies for delayed cerebral ischemia in aneurysmal subarachnoid hemorrhage. *Neurocrit Care* 2023;20:136–50. [CrossRef]
 40. Sen O, Caner H, Aydin MV, Ozen O, Atalay B, Altinors N, et al. The effect of mexiletine on the level of lipid peroxidation and apoptosis of endothelium following experimental subarachnoid hemorrhage. *Neurol Res* 2006;28:859–63. [CrossRef]
 41. Kumar V, Abbas A, Aster J. Robbins basic pathology. Elsevier 2017.
 42. Ayer RE, Zhang JH. Oxidative stress in subarachnoid haemorrhage: significance in acute brain injury and vasospasm. *Acta Neurochir Suppl* 2008;104:33–41. [CrossRef]
 43. Buzdağlı Y, Eyipınar CD, Kacı FN, Tekin A. Effects of hesperidin on anti-inflammatory and antioxidant response in healthy people: a meta-analysis and meta-regression. *Int J Environ Health Res* 2023;12:1390–405. [CrossRef]
 44. Hu P, Yan T, Xiao B, Shu H, Sheng Y, Wu Y, et al. Deep learning-assisted detection and segmentation of intracranial hemorrhage in noncontrast computed tomography scans of acute stroke patients: a systematic review and meta-analysis. *Int J Surg* 2024;63:3839–47. [CrossRef]
 45. Megyesi JF, Vollrath B, Cook DA, Findlay JM. In vivo animal models of cerebral vasospasm: a review. *Neurosurgery* 2000;47:448–60. [CrossRef]
 46. Neff S. In vivo animal models of cerebral vasospasm: a review. *Neurosurgery* 2000;47:794–5. [CrossRef]
 47. Marbacher S, Grüter B, Schöpf S, Croci D, Nevzati E, D'Alonzo D et al. Systematic review of in vivo animal models of subarachnoid hemorrhage: species, standard parameters, and outcomes. *Transl Stroke Res* 2018;9:1. [CrossRef]
 48. Ozdemir A, Ogden M, Kartal B, Ceylan AF, Yuksel U, Bakar B. Investigation of therapeutic effects of calcium dobesilate in cerebral hypoxia/reperfusion injury in rats. *Neurol Res* 2023;45:472–87. [CrossRef]
 49. Parhiz H, Roohbakhsh A, Soltani F, Rezaee R, Iranshahi M. Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models. *Phytother Res* 2015;29:323–31. [CrossRef]
 50. Pyrzynska K. Hesperidin: a review on extraction methods, stability and biological activities. *Nutrients* 2022;14:2387. [CrossRef]
 51. Choi SS, Lee SH, Lee KA. A comparative study of hesperetin, hesperidin and hesperidin glucoside: antioxidant, anti-inflammatory, and antibacterial activities in vitro. *Antioxidants (Basel)* 2022;11:1618. [CrossRef]
 52. Amiri H, Javid H, Hashemi SE, Reihani A, Esharham A, Hashemy SI. The protective effects of hesperidin as an antioxidant against quinolinic acid-induced toxicity on oligodendroglia cells: an in vitro study. *Mult Scler Relat Disord* 2024;82:105401. [CrossRef]
 53. Song B, Hao M, Zhang S, Niu W, Li Y, Chen Q, et al. Comprehensive review of hesperetin: advancements in pharmacokinetics, pharmacological effects, and novel formulations. *Fitoterapia* 2024;179:106206. [CrossRef]
 54. Han X, Zhang Y, Zhang L, Zhuang Y, Wang Y. Efficacy and molecular mechanisms of hesperidin in mitigating Alzheimer's disease: a systematic review. *Eur J Med Chem* 2025;283:117144. [CrossRef]
 55. Ma R, You H, Liu H, Bao J, Zhang M. Hesperidin: a citrus plant component, plays a role in the central nervous system. *Heliyon* 2024;10:e38937. [CrossRef]
 56. Watanabe T, Sasaki T, Asano T, Takakura K, Sano K, Fuchinoue T, et al. Changes in glutathione peroxidase and lipid peroxides in cerebrospinal fluid and serum after subarachnoid hemorrhage—with special reference to the occurrence of cerebral vasospasm. *Neurol Med Chir (Tokyo)* 1988;28:645–9. [CrossRef]
 57. Macdonald RL, Weir BK, Runzer TD, Grace MG, Poznansky MJ. Effect of intrathecal superoxide dismutase and catalase on oxyhemoglobin-induced vasospasm in monkeys. *Neurosurgery* 1992;30:529–39. [CrossRef]
 58. Macdonald RL, Weir BK, Runzer TD, Grace MG. Malondialdehyde, glutathione peroxidase, and superoxide dismutase in cerebrospinal fluid during cerebral vasospasm in monkeys. *Can J Neurol Sci* 1992;19:326–32. [CrossRef]
 59. Froehler MT, Kooshkabi A, Miller-Lotan R, Blum S, Sher S, Levy A, et al. Vasospasm after subarachnoid hemorrhage in haptoglobin 2-2 mice can be prevented with a glutathione peroxidase mimetic. *J Clin Neurosci* 2010;17:1169–72. [CrossRef]
 60. Krenzlin H, Wesp D, Schmitt J, Frenz C, Kurz E, Masomi-Bornwasser J, et al. Decreased superoxide dismutase concentrations (SOD) in plasma and CSF and increased circulating total antioxidant capacity (TAC) are associated with unfavorable neurological outcome after aneurysmal subarachnoid hemorrhage. *J Clin Med* 2021;10:1188. [CrossRef]
 61. Jani MS, Zou J, Veetil AT, Krishnan Y. A DNA-based fluorescent probe maps NOS3 activity with subcellular spatial resolution. *Nat Chem Biol* 2020;16:660–6. [CrossRef]
 62. Liu J, Hughes TE, Sessa WC. The first 35 amino acids and fatty acylation sites determine the molecular targeting of endothelial nitric oxide synthase into the Golgi region of cells: a green fluorescent protein study. *J Cell Biol* 1997;137:1525–35. [CrossRef]

63. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J* 2012;33:829–37. [CrossRef]
64. Mungrue IN, Husain M, Stewart DJ. The role of NOS in heart failure: lessons from murine genetic models. *Heart Fail Rev* 2002;7:407–22. [CrossRef]
65. Green SJ, Scheller LE, Marletta MA, Seguin MC, Klotz FW, Slayter M, et al. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. *Immunol Lett* 1994;43:87–94. [CrossRef]
66. Pluta RM. Dysfunction of nitric oxide synthases as a cause and therapeutic target in delayed cerebral vasospasm after SAH. *Acta Neurochir Suppl* 2008;104:139–47. [CrossRef]
67. Crobbeddu E, Pilloni G, Tardivo V, Fontanella MM, Panciani PP, Spena G, et al. Role of nitric oxide and mechanisms involved in cerebral injury after subarachnoid hemorrhage: is nitric oxide a possible answer to cerebral vasospasm? *J Neurosurg Sci* 2016;60:385–91.
68. Pluta RM, Oldfield EH. Analysis of nitric oxide (NO) in cerebral vasospasm after aneurysmal bleeding. *Rev Recent Clin Trials* 2007;2:59–67. [CrossRef]
69. Pluta RM. Delayed cerebral vasospasm and nitric oxide: review, new hypothesis, and proposed treatment. *Pharmacol Ther* 2005;105:23–56. [CrossRef]
70. Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochem J* 1994;298:249–58. [CrossRef]
71. Ahmad N, Ansari MY, Haqqi TM. Role of iNOS in osteoarthritis: pathological and therapeutic aspects. *J Cell Physiol* 2020;235:6366–76. [CrossRef]
72. Stuehr DJ. Mammalian nitric oxide synthases. *Biochim Biophys Acta* 1999;1411:217–30. [CrossRef]
73. Atkins GB, Jain MK. Role of Krüppel-like transcription factors in endothelial biology. *Circ Res* 2007;100. [CrossRef]

DENEYSSEL ÇALIŞMA - ÖZ

Şıçanlarda subaraknoid kanama sonrası serebral vazospazm üzerine hesperidinin nöroprotektif etkileri: Deneysel bir çalışma

AMAC: Subaraknoid kanama (SAK), yüksek morbidite ve mortalite oranlarıyla seyreden ciddi bir nörolojik acildir. Bu olumsuz sonuçların başlıca nedenleri vazospazm ve gecikmiş iskemidir. Doğal bir flavonoid olan hesperidin (HSP), güçlü antioksidan ve vazoprotektif etkilere sahiptir. Bu deneysel hayvan çalışmasında, HSP'nin SAK sonrası oksidatif stres ve vasküler yeniden yapılanma üzerindeki nöroprotektif rolü araştırılmış, özellikle Erk5-KLF2-eNOS sinyal yoluna odaklanılmıştır.

GEREÇ VE YÖNTEM: Çalışma 2021–2022 yılları arasında yürütüldü. Toplam 40 dişi Wistar albino şıçan beş gruba ayrıldı: Kontrol (G1, n=8), sham (G2, n=8), SAK + taşıyıcı (G3, n=8), düşük doz HSP (G4, n=8) ve yüksek doz HSP (G5, n=8). SAK modeli, cisterna magna'ya homolog kanın çift enjeksiyonu ile oluşturuldu. Subaraknoid kanamadan 48 saat sonra biyokimyasal belirteçler [Süperoksit Dismutaz (SOD), Katalaz (CAT), Glutatyon Peroksidaz (GPx), Nitrik Oksit (NOS)], baziler arter morfometrisi ve moleküler düzeyde Erk5, p-Erk5, KLF2 ve eNOS ekspresyonları değerlendirildi.

BULGULAR: Tedavi edilmeyen şıçanlarda (G3), SAK oksidatif stresi anlamlı düzeyde artırmış ve vasküler lümen çapını azaltmıştır. HSP ile tedavi edilen gruplarda (G4 ve G5) antioksidan enzim düzeylerinde (SOD, CAT, GPx) belirgin artış ve NOS düzeylerinde normale yakın değerler gözlenmiştir. Morfometrik analiz, HSP uygulanan gruplarda baziler arter lümen çapının anlamlı şekilde korunduğunu, duvar kalınlığında ise belirgin bir değişiklik olmadığını göstermiştir. Moleküler analiz sonuçları, Erk5-KLF2-eNOS yolunun yukarı regülasyonunu ortaya koyarak, vazodilatasyon ve oksidatif stresin azaltılmasında bu yolun rolünü desteklemiştir.

SONUÇ: Hesperidin, SAK'ye bağlı vazospazm ve oksidatif hasara karşı antioksidan kapasiteyi artırarak ve Erk5-KLF2-eNOS sinyal yolunu modüle ederek koruyucu etki göstermektedir. Bu bulgular, HSP'nin terapötik potansiyeline işaret etmektedir.

Anahtar sözcükler: Hesperidin; subaraknoid kanama; şıçan; vazospazm.

Ulus Travma Acil Cerrahi Derg 2025;31(12):1157-1167 DOI: 10.14744/tjtes.2025.52931