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Myricetin ameliorates cerebral ischemia/reperfusion damage in rat four vessel occlusion model: Histological, biochemical, molecular findings

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Abstract

Aim: Due to the loss of neurons and neural network functions in the brain, cerebral ischemia is the leading cause of permanent disability in adults all over the world. Antioxidants scavenge free radicals and protect tissues against oxidative damage caused by ischemia/reperfusion. This study aims to investigate the protective effects of myricetin, a powerful antioxidant, against cerebral ischemia/reperfusion injury.

Materials and Methods: There were 5 experimental groups in this study; control, sham, ischemia/reperfusion (I/R), Myricetin 3 (Myr 3), and Myricetin 6 (Myr 6), and 8 rats in each group. Four-vessel occlusion was made to create ischemia. The ischemia and subsequent reperfusion period were 30 minutes each. One hour before ischemia, 3 mg/kg of myricetin was given to rats in the Myr 3 group and 6 mg/kg of myricetin was given to rats in the Myr 6 group. GolgiCox and Caspase3 stainings were carried, SOD, and MDA levels were investigated, and TNF-α mRNA expression levels were measured in brain tissue.

Results: This study's results showed that; the number of neurons decreased drastically in the CA1region of the hippocampus in the I/R group. Distance between cells increased, and the neurons were spaced apart and randomly distributed. Neuronal loss in the hippocampus was reduced and the amounts of neurons rise in the Myr 3 group compared to the I/R group. A significant decrease was detected in the SOD values of the I/R group. Compared to the I/R group, the SOD values of Myr 3 and Myr 6 groups increased significantly. MDA level of the I/R group increased significantly compared to the control. MDA values of Myr 3 and Myr 6 groups decreased significantly when compared to the I/R group. TNF-α mRNA expression level was significantly higher in the I/R group. A significant reduction was observed in the mRNA expression level in the Myr 3 group.

Conclusion: In conclusion, the findings of this study suggest that myricetin is a promising agent for inhibiting the ischemia-induced cerebral inflammatory response and oxidative stress.

Introduction

Brain ischemia occurs when cerebral blood flow falls below the critical threshold. Ischemic stroke is an important cause of death in the world and causes significant disability. There is an increase in the frequency of thromboembolic events in the brain, especially with the acceleration of the atherosclerotic process with aging. This situation increases the risk of occlusion of cerebral blood vessels and ischemia [1].

Thrombolytic therapy is used in the treatment of cerebral ischemia. In this way, although blood flow to the tissue is restored, there is no functional improvement as desired. Replenishment of oxygen with reperfusion causes excessive free radical formation. Reperfusion elicits a series of inflammatory responses that result in tissue damage, including activation of neutrophils and platelets [2].

In particular, the generation of neutrophil-derived reactive oxygen species triggers ischemia and reperfusion damage [3]. When free radicals increase in the cell, basic structures such as proteins, lipids, and nucleic acids are dam-

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aged. The brain's cellular and metabolic changes cause some irreversible dysfunctions that may result in apoptosis, necrosis, and cell death [4].

Ischemia and the subsequent reperfusion period cause neurodegenerative lesions in the hippocampus, stratum, and CA1regions of the neocortex of the brain. It weakens spatial learning and memory [5]. GolgiCox staining is an effective method for staining mature neurons in regions of the hippocampus such as CA1, CA3, dentate gyrus [6]. It allows the determination of neuronal damage. Caspase 3 staining is used to detect apoptosis due to ischemia-reperfusion injury in tissue [7]. In this study, we aimed to detect neuron damage in CA1 region of hippocampus with GolgiCox and Caspase 3 staining.

ROS formation and increase in mitochondrial MDA following ischemia/reperfusion damage increase lipid peroxidation, which accelerates the imbalance between oxidative stress and antioxidants. The SOD enzyme works as an endogenous antioxidant in the tissue and catalyzes superoxide anions to H2O2 (hydrogen peroxide). Then, H_2O_2 is catalyzed to H_2O and O_2 by peroxidases such as catalase and inactivated. In this way, oxidative damage is tried to be prevented. However, the antioxidant defense system in the tissue is insufficient against the formation of too much superoxide. Therefore, the formation of proinflammatory cytokines such as TNF-α, IL-1β, and, IL-6 increases to prevent ischemia/reperfusion damage and a cytokine storm occurs. Studies have shown that increased levels of these cytokines are associated with increased mortality, acute respiratory distress syndrome, and multiple organ failure [8].

Many studies have used these parameters to determine ischemia reperfusion. MDA [9] has been used to determine lipid peroxidation, SOD [10], CAT [11], and TNF-a [12] has been used to determine oxidative stress after ischemia-reperfusion injury. In this study, the changes in MDA, CAT, SOD, and TNF-α mRNA expression levels, which are indicators of ischemia/reperfusion damage, in brain tissue were investigated. In addition, changes in brain tissue due to ischemia/reperfusion damage were also examined.

There are many studies on the protective properties of antioxidants in the prevention of ischemia/reperfusion injury. Antioxidants are substances that eliminate free radicals, prevent damage to cell membranes and DNA, and reduce cell death. Studies on ischemia/reperfusion injury have shown that antioxidants reduce leukocyte-mediated injury, improve microvascular perfusion, and reduce tissue damage after ischemia [13].

Myricetin is a polyhydroxyflavonol compound. This is abundant in black tea, fruits such as strawberries, onions, garlic, cabbage, apples, cherries, grapes, some vegetables, and medicinal plants. Its chemical formula is C15H10O8 (3,5,7,3´,4´,5´-hexahydroxyflavonol) [14].

Myricetin is a flavonoid with potent antioxidant and anti-inflammatory properties [14]. It has been shown to contribute to the recovery of neuroinflammation and oxidative damage in the brain after ischemia .The use of myricetin in the treatment of ischemic neuro injury has certain advantages: it (a) decreases the production of amyloid, (b) protects the development of neurofibrillary tangles, (c) decreases neuroinflammation, (d) acts as a powerful antioxidant, (e) increases acetylcholine level, (f) induces protective autophagy after ischemia, and (g) can be taken in relatively high quantities without side effects [14-16]. Therefore, myricetin has significant potential to prevent stroke and I/R injury. Myricetin is also known to have anticarcinogenic, antimutagen, antiinflammatory, and antidiabetic properties [17]. In some studies, it has been shown that myricetin has an antiproliferative effect on esophageal, leukemia, lung, prostate, and thyroid cancer cells [18,19].

Various myricetin doses have been used in cerebral ischemia and neurodegenerative studies. In the cerebral ischemia study of Sun et al. [20], doses of 1 mg/kg, 5 mg/kg, and 25 mg/kg were tried and significant neuroprotective effects were observed in the group administered 25 mg/kg. Ye et al. [21] administered 5 mg/kg myricetin to rats in a similar study. In the experimental stroke study of Wu et al. [22] 10 and 20 mg/kg, and in the cerebral ischemia study of Al-Husein et al. [23], 50 mg/kg of myricetin was administered to rats. The high doses administered in these studies far exceed the amount of myricetin in the daily diet. In our study, doses of 3mg/kg and 6 mg/kg of myricetin were tried. We kept the dose of myricetin low because these doses are more appropriate for the amount we can get in the daily diet [25]. Thus, we aimed to determine whether it is possible to protect against cerebral ischemia with a daily nutrition program.

The aim of this study is to investigate whether myricetin has a protective effect against the damage in rats with cerebral I/R damage. For this purpose, MDA, SOD, CAT, and, TNF-α mRNA expression levels, which are markers of ischemia/reperfusion damage in tissue, were determined and tissue histology was examined.

Materials and Methods

Animal experiments of this study were conducted in the Kütahya Health Sciences University Faculty of Medicine Experimental Animal Breeding Research and Application Center laboratory. Necessary ethic permissions for animal experiments were obtained from Kütahya Health Sciences University Experimental Animals Ethics Committee (No: 2017.06.03). In the selection of experimental animals in this study, sampling was done as probable, and the simple random sampling method was used.

In our study, there were 5 groups and 8 rats in each group. There is no universal rule for determining the number of animals used in experiments. In our study, we followed the 3R (Replacement, Reduction, Refinement) rule in determining the number of experimental animals. In Doğan and Doğan's study [25], the number of animals to be used in the experiments was determined according to the resource equality method, and it was found that the sample size should be maximum 5 per group in a 5-group study.

However, since this number was not sufficient for statistical analysis, we used 8 animals in each group for statistical reliability in our study.

Eksperimental groups

Forty male Sprague Dawley rats were used in the study. Groups were created as follows;

Control group (n=8): No treatment was applied to the rats in this group.

Sham group (n=8): Surgical procedure was applied to the rats in this group, but ischemia/reperfusion damage was not created. 1 ml of saline was given one hour before the surgical procedure.

Ischemia/reperfusion (I/R) group (n=8): Half an hour of ischemia and half an hour of reperfusion were applied to the rats in this group.

Myricetin 3 mg/kg + I/R (Myr 3) group (n=8): 3 mg/kg myricetin was injected intraperitoneally into the rats in this group one hour before the ischemia. (Myricetin; TCI- Tokyo Chemical Industry, Japan (M2131), Cas number:529-44-2 (>97.0%), MW=318.24).

Myricetin 6 mg/kg + I/R (Myr 6) group (n=8): 6 mg/kg myricetin was injected intraperitoneally into the rats in this group one hour before the ischemia.

Experimental procedure-induction of cerebral ischemia/reperfusion

In our study, the four-vessel occlusion model was used to induce ischemia [26]. After anesthesia and analgesia, both Arteria vertebralis were cauterized along the foramen alaris of C1 with bipolar electrocautery. 24 hours after cautery application, a midline incision of the neck was made and both Arteria carotis communis were kept closed for 30 minutes with the Vasco Bulldog clamp. Then, the clamps were removed and reperfusion was applied for 30 minutes. After ischemia/reperfusion, the rat brains were removed with a wide craniotomy. Brain tissues were divided into three parts. The first piece was fixed with a 10% formaldehyde solution for histological examination. The second and third pieces were stored at -80 ° C for biochemical and molecular studies.

Histological examination

Brain tissues were fixed with 10% formaldehyde solution and were embedded in paraffin after the routine tissue fixation process and sectioned at 5-10 µm with a microtome. GolgiCox and Caspase3 staining were applied to the tissues. Stains were examined under a microscope, and the CA1 region of the hippocampus was photographed and analyzed with an image analysis program.

Biochemical analyses

For biochemical analysis, brain tissues, stored at -80 ° C, were homogenized at 8000 rpm for 5 minutes with a homogenizer in a chilled sodium phosphate buffer at pH 7.4, 50µM and containing 0.25M sucrose. The homogenates were precipitated by centrifugation at 10,000 rpm, at +4°C, for 30minutes and the supernatant was used to determine SOD and CAT enzyme activities, MDA levels, and total protein quantification.

Determination of malondialdehyde (MDA) level

For the lipid peroxidation index; the MDA level was measured by the double heating method [27]. In this method, thiobarbituric acid (TBA) reacts with MDA and pink color is formed. This color is measured spectrophotometrically at 532 nm. MDA concentrations were calculated with the aid of the standard table of the MDA-TBA complex [28].

Determination of catalase (CAT) activity level

CAT activity was measured spectrophotometrically by observing changes in sample and blank absorbance at 240 nm for one minute [29]. Determination of superoxide dismutase (SOD) activity level The method of determining SOD activity depends on spectrophotometric measurement of the inhibitory effect of SOD on the autoxidation of 6-hydroxydopamine (6-OHDA) [30]. Spectrophotometric measurements were made at 490 nm until the 60th second of oxidation. Because the autoxidation rate curve is constant in the first minute. The results were calculated as U/mg protein by determining the amount of protein.

Protein quantification

The protein concentration of tissue homogenates was calculated as mg/ml using bovine serum albumin according to the method of Lowry et al. [31].

Molecular analyses

For the molecular study, brain tissues were homogenized with a homogenizer before RNA isolation from the brain part stored at -80 ° C. Then the brain tissues were centrifuged. mRNA was isolated from the supernatant using the High Pure RNA Tissue Kit - Version 09 (Roche). Subsequently, cDNA was obtained with Transcriptor First Strand cDNA Synthesis Kit-Version 6.0 (Roche). Sample cDNAs were diluted at 1:10 with PCR-grade water. The amount of mRNA expression of the TNF-α gene was determined by RT-qPCR using the FastStart Essential DNA Green Master (Roche) in accordance with the kit procedures. Specially produced primers were used for this. The base sequence of the primers is given below.

Rat TNF-α (Forward): 5' TGAACTTCGGGGTGATCG 3'

Rat TNF-α (Reverse): 5' GGGCTTGTCACTCGAGT TTT 3'

Housekeeping gene

β-Actin gene was used as the housekeeping gene for normalization. The specific production primer sequences of the β-Actin gene used are given below.

β-Actin (Forward): 5' CCCGCGAGTACAACCTTCT 3'

β-Actin (Reverse): 5' CGTCATCCATGGCGAACT 3'

Statistical analyses

The biochemical results obtained were analyzed using IBM SPSS Statistics Version 20 package program. Continuous variables are expressed as mean±standard deviation. ANOVA test was used for intergroup comparisons and the Post Hoc test was used for intragroup comparisons. A p-value of <0.05 was considered statistically significant. In the molecular analysis, CP values and melting curves of the samples were determined by RT-qPCR analysis. The data obtained were analyzed using LightCycler 480 Instrument Software Version 1.5.1.

Table 1. Comparison of the biochemical analysis results of the groups.

Parameters	Control (n=8)	Sham (n=8)	I/R (n=8)	Myr 3 (n=8)	Myr 6 (n=8)
CAT (U/mg. protein)	0.98±0.32[a]*	0.81±0.27[a]	0.93±0.64[a]	0.88±0.22[a]	1.02±0.57[a]
SOD (U/mg. protein)	5.53±0.72[a]	5.43±0.25[a]	4.44±1.08[b]	6.93± 18[c]	4.96 ± 0.76[a]
MDA (nmol/mg. protein)	9.34±0.2[a]	10.31±0.6[a]	17.32±0.1[b]	13.65±0.41[c]	17.32±0.13[d]
T.Protein (mg/ml)	88.5±0.2[a]	91.3±0.8[a]	90.8±0.7[a]	93.4±0.3[a]	89.2±0.6[a]

Results are given as mean ± SD.

* : The letters above the numbers indicate statistical significance. Numbers shown with the same letters on the horizontal plane are not statistically different from each other (p> 0.05), while the numbers shown with different letters are statistically significantly different from each other (p <0.05).

Results

Histological examination results

A nucleus with prominent round or oval shaped nucleolus surrounded by a homogeneously stained cytoplasm was observed in the center of the pericarion in most neurons in the CA1region of the hippocampus in the control and sham groups. (Figure 1). It was observed that hippocampal neurons were arranged in a certain order in the sham and control groups.

CA1 neurons of the rat hippocampus in the I/R group showed morphological changes consistent with degeneration. Many neurons were stained darkly due to the condensation of their cytoplasm and nuclei in the I/R group. This is an indicator of degeneration. Significant loss of CA1neurons was determined in the I/R group. Because the distance between cells increased, the neurons were spaced apart and randomly distributed and showed an irregular order (Figure 2).

Neuron damage in the hippocampus decreased and the number of neurons increased statistically significantly in the Myr 3 group compared to the I/R group. In the Myr 6 group, neuronal damage in the hippocampus was partially reduced and the amount of neurons increased, but there was no significant difference in histological examination

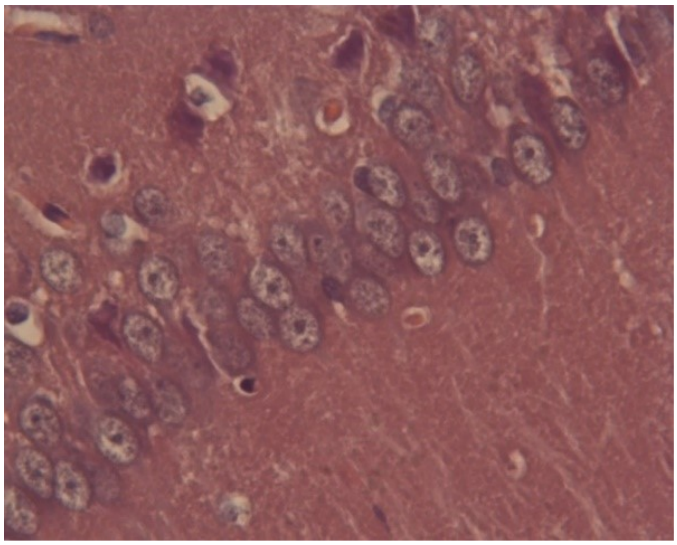

Figure 1. CA1 region of the hippocampus in the control group (staining of neurons are homogeneous, large and round-shaped nuclei, prominent nucleoli).

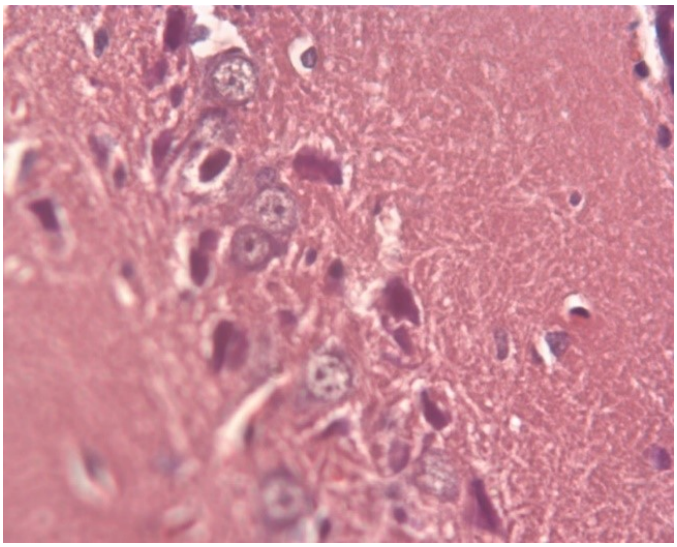

Figure 2. CA1 region in the hippocampus in the I/R group (dark and shrunken neurons with many pycnotic nuclei, a large distance between neurons due to loss of neurons, irregularly distributed neurons).

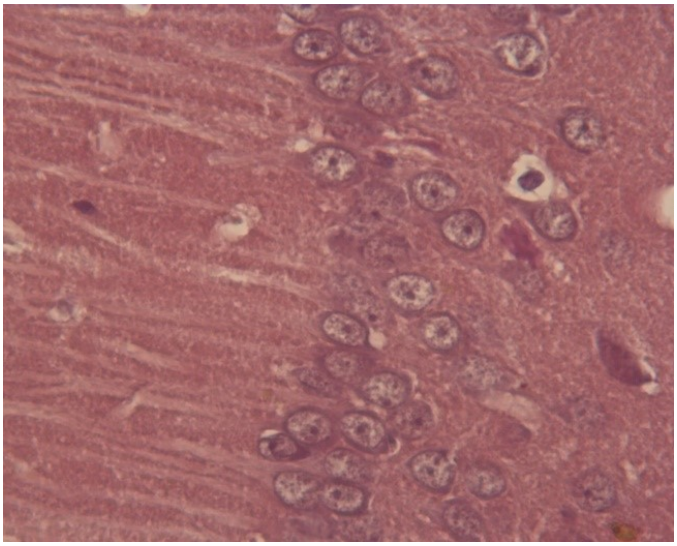

Figure 3. CA1 region in the hippocampus in the Myr 3 group (Few damaged neurons and neuronal loss were observed).

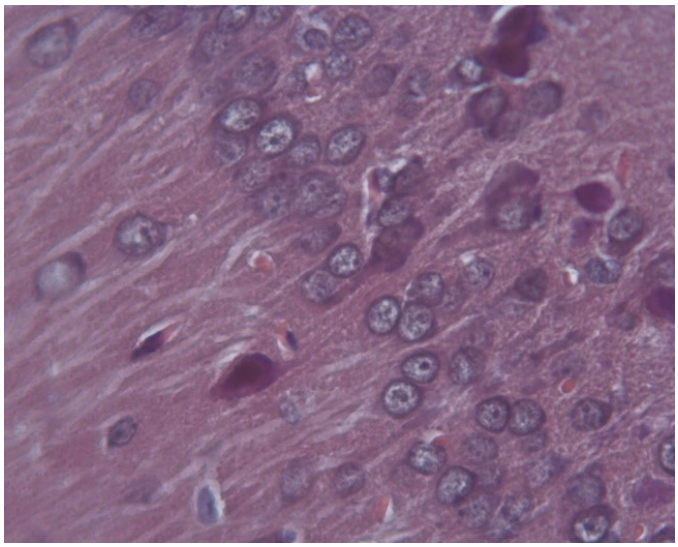

Figure 4. CA1 region in the hippocampus in the Myr 6 group (Few hyperchromatic neurons and neuronal loss were observed).

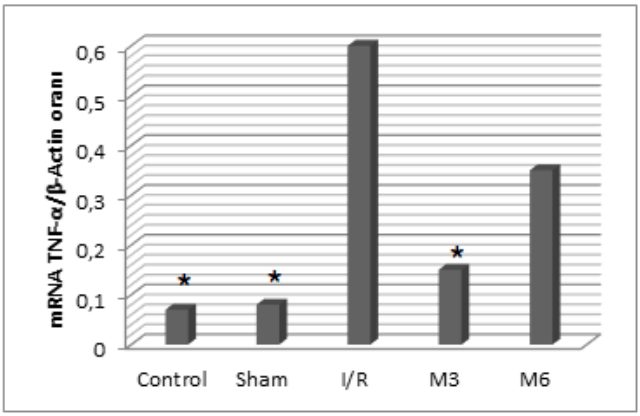

Figure 5. TNF-α mRNA expression levels of the groups. (* compared to I/R group $p<0.05$).

compared to the I/R group. (Figures 3 and 4).

Biochemical analysis results

Values obtained as a result of biochemical analysis are given in Table 1.

There was no significant difference between the control and sham groups in terms of the CAT values. There was a slight decrease in the I/R group in the CAT values compared to the control, but this value is not statistically significant. There was a slight increase in the Myr 6 group compared to the other groups. But this is also not significant.

There were no significant differences between the SOD values of the control and sham groups. A significant decrease was detected in the SOD values of the I/R group compared to the control. Compared to the I/R group, the SOD values of Myr 3 and Myr 6 groups increased significantly. The SOD values of the Myr 3 group were significantly higher than the I/R group and the control group. Although the SOD values in the Myr 6 group were slightly lower than in the control, this decrease was not statistically significant.

The MDA values were statistically significantly increased in the I/R group compared to the control. MDA values of Myr 3 and Myr 6 groups decreased significantly when compared to I/R. The decrease in the MDA value of the Myr 6 group was closer to the control. There is also a statistically significant difference between the Myr 3 and Myr 6 groups.

Molecular analysis results

RNA was isolated from brain tissues and TNF-α mRNA expression levels were determined by RT-qPCR. According to the results, there was no difference between the control group and the sham group in terms of mRNA expression values in brain tissue. TNF-α mRNA expression was found to be significantly higher in the I/R group, which was not given myricetin by applying only ischemia/reperfusion, compared to control and sham groups. A significant reduction was observed in the amount of mRNA expression in the Myr 3 group, in which 3 mg/kg myricetin was applied as a preservative before ischemia/reperfusion when compared with the I/R group. A slight reduction in the amount of TNF-α mRNA expression was detected in Myr 6 group compared to the I/R group and this decrease was not statistically significant.

TNF-α mRNA expression levels of the groups are given in Figure 5.

Discussion

The brain is very susceptible to oxidative stress damage. Because, the oxidative metabolic activity rate of the brain is high, its antioxidant capacity is relatively low, and neuronal repair is insufficient. Due to the loss of neurons and neural network functions in the brain, cerebral ischemia is the leading cause of permanent disability in adults all over the world. Ischemia reduces the patient's life quality and increases the global medical burden. Although thrombolytic and antidepressant agents are used in the treatment of ischemia and its subsequent neuropsychiatric symptoms, the treatment of most stroke cases is not satisfactory.

Reducing oxidative stress is a potential strategy to prevent I/R damage. Studies have shown that high reactive oxygen species levels and oxidative stress damage after ischemia/reperfusion can be reduced by antioxidant treatment [32]. Flavonoids may play a protective role in many pathological mechanisms due to their anti-inflammatory and antioxidant properties [33].

The antioxidant capacity of myricetin is stronger than other flavonoid types because it has more phenolic hydroxyl groups [34]. With this feature, myricetin prevents oxidative stress and plays a protective role on cells by inhibiting the formation of reactive oxygen species and increasing the effectiveness of antioxidant enzymes [20,22,35].

Some studies indicate that the efficacy of myricetin appears to be dose-dependent. In the study of Qiu et al. [32], antioxidant effects were observed in the 5µM myricetin applied group, while prooxidant effects were observed in the 10 and 20 µM applied groups.

Studies have shown that cerebral ischemia causes acute major neuronal loss in the CA1region of the hippocampus and cerebral cortex in humans and animals [36]. In a previous study, 10 days after ischemia/reperfusion, brain tissues were stained with cresyl violet. In the brain tissues, concentrated, pyknotic and shrunken neurons have been determined in the CA1 region of the hippocampus [37]. Janyou et al. [37] observed that neurons in the cortex region of the brains, which were removed 24 hours after ischemia/reperfusion, became composed of a pycnotic nucleus and a vacuole surrounding it.

Cytokines are major inflammatory mediators with well-known roles in ischemic injury, too [39]. In some studies, the extent of ischemic damage to tissue has been associated with the presence of cytokines released in the early stage of ischemia [40].

Shah et al. [41] detected marked necrosis and occlusion of blood vessels in the brain after 30 minutes of cerebral ischemia and 1 hour of reperfusion. In the study of Chandrashekhar et al. [42], severe and moderate brain damage caused by I/R was demonstrated by histopathological scores. The study found that myricetin treatment significantly improved brain damage. On histopathological examinations, normal brain histology and mild damage were observed in the myricetin group [42]. Sun et al. [43] showed that myricetin treatment in a rat intestinal I/R model significantly reduced the damage score in the histopathological examination in a dose-dependent. According to the findings of Ramezani et al. [44], myricetin showed a neuroprotective effect by increasing the number of neurons in the CA3 region of the hippocampus in rats with Alzheimer's disease. Myricetin is a lipophilic compound and can cross the blood-brain barrier [45]. In this way, it can have a healing effect in chronic brain damage in neurodegenerative diseases.

In this study, ischemia-reperfusion-related degeneration in the CA1 region of the hippocampus decreased in the myricetin groups. Myricetin prevented tissue degeneration by increasing the release of antioxidant enzymes (SOD), preventing lipid peroxidation (MDA reduction), and reducing the release of cytokines that cause damage to brain tissue.

The generation of reactive oxygen species is the main factor associated with I/R injury [46]. Under normal conditions, increased levels of reactive oxygen species can be reduced by using antioxidant systems. When these systems are overloaded, the amount of reactive oxygen species increases too much. For this increase, the capacity of the antioxidant defense system is not sufficient and oxidative stress occurs. These systems include enzymes such as SOD, and CAT [47].

As a result of this study, SOD values, which decreased due to ischemia-reperfusion, increased in myricetin groups. This shows that myricetin activates the antioxidant defense system and contributes to the prevention of cerebral damage.

MDA is a stable end product of degradation due to lipid peroxidation produced by reactive oxygen species. It is an indicator of lipid peroxidation in I/R injuries [48].

In our study, MDA values, which were increased after ischemia-reperfusion, decreased significantly in myricetin groups.

The previous studies support the findings obtained in our study. In the study of Qiu et al. [32] on myocardial I/R damage, 5µM myricetin was given to rats as a protective before 45 minutes of I/R administration. They found that the SOD level increased in the myricetin group compared to the I/R group, while the MDA level decreased [32]. In the testicular I/R study of Öztürk et al. [49], they applied 2 hours of ischemia and 2 hours of reperfusion to rats. They gave 25 and 50 mg/kg doses of myricetin 30 minutes before I/R as a prophylactic. In the study, SOD level decreased and MDA level increased in the I/R group. It was observed that SOD increased significantly and MDA decreased compared to I/R in the myricetin group. This decrease was greater in the high-dose myricetin group [49].

Similarly, in the study of Sun et al. [20], with the application of myricetin in the middle cerebral artery occlusion model, the SOD level, which had decreased as a result of ischemia, increased and the increased MDA level decreased.

This data shows that myricetin, a powerful antioxidant, neutralizes free radicals caused by ischemia/reperfusion, prevents neuronal loss in the brain, and thus prevents the brain from being damaged.

One of the main processes of ischemic neurodegeneration is neuroinflammation too [50]. In the case of ischemia and hypoxia, the release of inflammatory cytokines (TNF-α, CRP, IL-1β, IL-6, PGE) from endothelial tissues increases [39,51]. TNF-α exacerbates the neuroinflammatory response in inflammation and increases cell and brain damage.

According to the results of our study, compared to the I/R group, a significant decrease was observed in the expression levels of TNF- α in the myricetin groups.

Myricetin, with its anti-inflammatory activity, alleviates the damage caused by these factors by reducing the release of anti-inflammatory factors in the brain [52,53].

In the study of Ye et al. [21], the levels of TNF-α, IL-6, and IL-1β cytokines in the brain tissue increased significantly after cerebral ischemia induced by the middle artery occlusion model. Application of myricetin as a protective significantly suppressed the rise of these values [21]. In the study of Sun et al. [43], myricetin reduced the increased TNF-α, IL-6, and IL-1β gene expressions in rats with cerebral ischemia with the middle cerebral artery occlusion model. In a study demonstrating the cardioprotective effect of myricetin against I/R injury, myricetin administration as a preservative significantly reduced TNF-α, IL-6, and CRP inflammatory factors compared to the I/R group [32].

Conclusion

Oxidative stress is the main phenomenon in brain neurodegeneration after ischemia. In this study, myricetin reduced oxidative stress by its antiinflamatuar and antioxidant features, and it showed a neuroprotective effect. With myricetin administration, MDA levels decreased significantly and SOD levels increased in rats. In this way, it reduced cerebral ischemia damage by regulating oxidative stress. At the same time, the increase in SOD indicates

that the decrease in total antioxidant capacity due to ischemia is regulated. Histological and molecular studies also support them. With the administration of myricetin, neuronal injury in the hippocampus decreased and the number of neurons increased. The decrease in the level of TNF-α, an anti-inflammatory factor, is also an indicator of the anti-inflammatory property of myricetin. According to the data of this study, we can say that myricetin is a versatile active ingredient. Clinical efforts to date have focused on a variety of antioxidant strategies aimed at scavenging reactive oxygen species to achieve functional recovery following reperfusion. In this regard, This study's findings suggest that myricetin is a promising agent for inhibiting the ischemia-induced cerebral inflammatory response and oxidative stress. Additionally, myricetin is safe as a pro-health ingredient that is commercially available easily and inexpensively, and can be taken with many foods. In conclusion, this study provides evidence for the potential benefits of myricetin in the prophylactic treatment of ischemia and other neurodegenerative disorders.

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Declaration of interest
The authors report no conflicts of interest.

Ethics approval
Animal experiments of this study were conducted in the Kütahya Health Sciences University Faculty of Medicine Experimental Animal Breeding Research and Application Center laboratory. Necessary ethic permissions for animal experiments were obtained from Kütahya Health Sciences University Experimental Animals Ethics Committee (No: 2017.06.03).

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