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*RESEARCH ARTICLE*

# POLYDATIN, A HERBAL BIOFLAVONOID, IS PROTECTIVE AGAINST CEREBRAL ISCHEMIA-REPERFUSION INJURY: MOLECULAR, BIOCHEMICAL AND HISTOLOGICAL DATA

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## ABSTRACT

This study aims to research the protective effects Polydatin have against cerebral ischemia/reperfusion damage. Polydatin is a natural polyphenic phytoalexin and which has strong antioxidant properties.
In the present study, 5 groups were prepared as control, sham, ischemia/reperfusion (IR), Polydatin 30 (Pol 30), and Polydatin 60 (Pol 60). The four-vessel occlusion model was used to induce ischemia. Polydatin was injected intraperitoneally 30 minutes before ischemia. Hematoxylin Eosin staining were applied for histopathological study, SOD, CAT, and MDA levels determined, and TNF-α mRNA expression levels were measured by the RT-qPCR technique in brain tissue. According to the results, a serious loss of neurons in the CA 1 region of the hippocampus was observed in the IR group. Neuronal damage in the hippocampus decreased and the number of neurons increased significantly in the Pol 60 group compared to the IR group. CAT and SOD levels were reduced, and the MDA level rose in the IR group. In Pol 60 and Pol 30 groups, an increase was observed in the CAT and SOD levels, a decrease was observed in the MDA and total protein levels compared to the IR group. The amount of TNF-α mRNA expression in the brain tissues of the IR group was significantly higher compared to the control group. In the Pol 60 group, mRNA expression level decreased significantly compared to the IR group. In conclusion, the increase in MDA, decrease in SOD and CAT values, increase in TNF-α gene mRNA expression, and histological damage in the brain because of cerebral ischemia/reperfusion in rats were restored to normal levels with 30 and 60 mg/kg polydatin administration as protective before ischemia. Especially at 60 mg/kg polydatin supplementation with antioxidant properties has a neuroprotective effect against oxidative stress damage caused by cerebral ischemia/reperfusion.

**Key Words:** *Cerebral Ischemia, Reperfusion, Polydatin, Protective Effect.*

## 1. INTRODUCTION

Cerebral ischemia occurs with a temporary or permanent decrease in cerebral blood flow as a result of thrombotic or thromboembolic arterial occlusion. Currently, thrombolytic drugs are used to provide cerebral perfusion and there is no other approved treatment [1]. In general, thrombolytic therapy ameliorates the acute effect of ischemia by regulating blood flow, but this treatment does not provide a significant improvement in motor and cognitive impairments [2]. For this reason, it is necessary to investigate the mechanisms of cerebral ischemia formation and to identify potent neuroprotective agents that can be used after ischemia occurs.

Reperfusion occurs after thrombolytic therapy. Oxygen is restored by reperfusion, making this situation worse because a large amount of reactive oxygen species are formed with oxygen, and irreversible damage occurs in the cell [3]. Oxidative damage, inflammations, and apoptosis develop in cerebral ischemia/reperfusion damage. Therefore, previous research has indicated that antioxidant, anti-apoptotic, and anti-inflammatory agents can be used as a protective agent against cerebral ischemia/reperfusion injury [4]. In this study, we examined the protective effects of polydatin, which has a strong antioxidant effect, against cerebral ischemia/reperfusion injury.

Polydatin is a natural polyphenic phytoalexin derived from the roots of a Chinese plant called *Polygonum cuspidatum*. Polydatin is also found in foods we consume daily, such as grapes, peanuts, cocoa products, and chocolate [5-9]. Its chemical formula is $C_{20}H_{22}O_8$ (3, 4', 5 – trihydroxy stilbene – 3 – β – mono – D – glucoside). It is the glycoside form of resveratrol and is also known as Piceid. *Polygonum cuspidatum* has been used in Chinese medicine since ancient times as an antitussive, antiasthmatic, expectorant and for blood lipid improvement, high cholesterol, and hypertension [10-12]. However, with a detailed study of its chemical extraction, it has been found to have some important effects, such as antimicrobial [13], anti-inflammatory [14], antioxidative [15-18], hepatoprotective [19, 20], anticancer [21], antiapoptotic effect [22], scavenging free radical, increasing antioxidant production, regulating immune functions [23], healing ischemic damage in some organs such as the heart, lungs [24,25], kidneys [26], and brain [2, 14, 27].

Previous research showed that polydatin has a protective effect against injury caused by an acute shock in neurons, smooth muscle cells, and erythrocytes [28]. It has also been determined that polydatin prevents sepsis-induced multiple organ dysfunction syndrome which causes mitochondrial injury in the lung, liver, kidney, and intestines [29-31]. In a study, it was determined that polydatin can cross the blood-brain barrier [14]. With its feature to pass the blood-brain barrier, polydatin can prevent cerebral ischemia-reperfusion damage. In inflammation models in previous studies, it was indicated that polydatin inhibits some pro-inflammatory cytokines [32]. It also decreased MDA and increased SOD and CAT thus it improved oxidative stress parameters [33].

This study aimed to determined the effects of polydatin on the levels of MDA, SOD, and catalase, which are oxidative stress parameters in ischemia-reperfusion injury. We also investigated the mRNA expression level of the TNF-α gene.

MDA is a lipid peroxidation marker. The reperfusion process after ischemia is very favorable for lipid peroxidation and the formation of new free radicals. [34]. Due to lipid peroxidation, ATPase activity decreases and the synthesis of vital proteins is inhibited. As a result, proteolytic enzymes and mitochondrial matrix enzymes are released and cellular damage occurs. In this case, the antioxidant defense system (SOD, catalase) plays a very important role in preventing neuronal death. [35].

Studies show an increase in the synthesis of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 after ischemia. There is a correlation between the increase in TNF-α, IL-1β, and IL-6 levels as a result of ischemia/reperfusion, an increase in mortality, acute respiratory distress syndrome, and increased risk of multiple organ failure [36]. The pathological process driven by increased intracellular $Ca^{+2}$ during ischemia/reperfusion is the formation of calcium pyrophosphate and uric acid. This process sends signals that activate inflammatory agents such as TNF-α and IL-1β (interleukin-1β), which exacerbate ischemia-reperfusion injury. These cytokines in turn activate transcription factors such as NF-κB to increase the expression of other cytokines and chemokines. Thus, it fires a cytokine storm that will trigger further cell damage with deep inflammation [37].

This study focuses on the free radical scavenging and inflammatory cytokine inhibitory effects of polydatin. For this purpose, we investigated the neuroprotective effects of polydatin, a new agent that can be used prophylactically against ischemia-reperfusion injury.

## 2. MATERIAL AND METHOD

Animal experiments of this study were conducted in the Kütahya Health Sciences University Faculty of Medicine Experimental Animal Breeding Research and Application Center laboratory. Ethical approval was token for animal experiments from the Animal Experiments Local Ethics Committee of Kütahya Health Sciences University Faculty of Medicine (No: 2019.03.04).

### 2.1. Experimental Groups

**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 (IR) group (n=8):** Half an hour of ischemia and half an hour of reperfusion were applied to the rats in this group.

**Polydatin 30mg/kg + IR (Pol 30) group (n=8):** 30mg/kg polydatin was injected intraperitoneally to the rats in this group one hour before the ischemia. (PD ($C_{20}H_{22}O_8$, MW: 390.38, purity ≥ 95%), Pub Chem Substance ID 329750984, Sigma- Aldrich Co. LLC. St. Louis, MO, USA).

**Polydatin 60 mg/kg + IR (Pol 60) group (n=8):** 60mg/kg polydatin was injected intraperitoneally to the rats in this group one hour before the ischemia.

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**2.2. Experimental Procedure-Induction of Ischemia/Reperfusion**

In our study, the four – vessels occlusion model was applied to induce ischemia. After anesthesia and analgesia, an incision was made in the midline of the dorsal of the neck, and both *Arteria vertebralis* were cauterized along the foramen alaris of C1 with bipolar electrocautery (Fig. 1). 24 hours after cautery application, midline incision of the neck was made. After superficial microdissection was performed, it was advanced towards the right and left carotid arteries (*Arteria carotis communis*, ACC) with deep microdissection. When the trachea was seen, the paratracheal muscles were dissected, and it was reached to ACC and both ACCs were kept closed for 30 minutes with the Vasco Bulldog clamp (Fig. 2). Then, the clamps were removed and reperfusion was applied for 30 minutes.

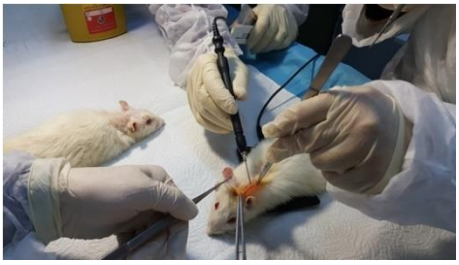

**Figure 1.** Electrocautery application (24 hour before ischemia).

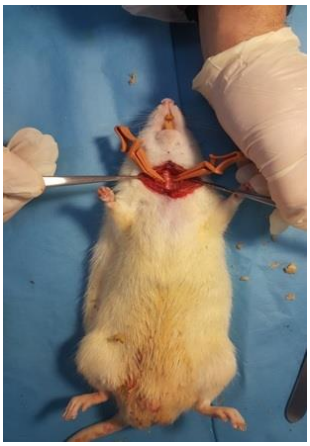

**Figure 2.** Ischemia application (ACCs were kept closed for 30 minutes with the Vasco Bulldog clamp).

After ischemia and reperfusion, the rats were sacrificed and brains were removed with craniectomy. Brain tissues were divided into three parts. The first piece was kept in a 10% formaldehyde for histological examination. The second and third pieces were kept at -80˚C until biochemical and molecular examinations were performed.

**2.3. Histopathological Examination**
Brain tissues were fixed with 10% formaldehyde solution. Then brain tissues were embedded in paraffin after the routine tissue fixation process and sectioned at 5-10µm with a microtome. Hematoxylin Eosin (H&E) staining were applied to the tissues. Stains were examined under a microscope, the CA1 region of the hippocampus was photographed and analyzed with an image analysis program.

**2.4. Biochemical Analyses**
For biochemical analysis, brain parts that were properly extracted from rats were stored at -80˚C. Before analyses, brain tissues were homogenized at 8000 rpm for 5 minutes with a homogenizer in a chilled sodium phosphate buffer at pH7.4, 50 µM containing 0.25 M sucrose. The homogenates were precipitated by centrifugation at 10,000 rpm at +4°C for 30minutes and the supernatant was used to define SOD, CAT, and MDA levels, and protein quantification.

**2.4.1. Determination of malondialdehyde (MDA) level**
MDA levels were defined using the double heating method of Draper and Hadley [38]. In this method, thiobarbituric acid (TBA) reacts with MDA and a pink color is formed. This color was measured spectrophotometrically at 532 nm. MDA concentrations were calculated with the aid of the standard table of the MDA-TBA complex [39-42].

**2.4.2. Determination of catalase (CAT) activity level**
CAT activities were determined using the Aebi method [43]. CAT enzyme activities were measured spectrophotometrically by observing changes in sample and blank absorbance at 240 nm for one minute.

**2.4.3. Determination of superoxide dismutase (SOD) activity level**
SOD levels were detected depending on spectrophotometric measurement of the inhibitory effect of SOD on the autoxidation of 6-hydroxydopamine (6-OHDA) [44, 45]. Spectrophotometric measurements were made at 490nm 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.

**2.4.4. Protein quantification**
The protein concentration of tissue homogenates was calculated as mg/ml using bovine serum albumin by method of Lowry et al. [46].

**2.4.5. Statistically analysis**
The results obtained were analyzed using IBM SPSS 20 package program. While ANOVA test was used for intergroup comparisons, the Post hoc test was used for intragroup comparisons and $p < 0.05$ was statistically significant.

**2.5. Molecular Analyses**
For the molecular study, brain tissues were homogenized with a homogenizer before RNA isolation from the brain tissue stored at -80˚C. Then the brain tissues were centrifuged, and mRNA was isolated

from the supernatant using the High Pure RNA Tissue Kit-Version09 (Roche). Subsequently, cDNA was obtained with Transcriptor First Strand cDNA Synthesis Kit-Version6.0 (Roche). Sample cDNAs were diluted 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, for which specially produced primers were used. The base sequence of the primers is given below. CP values and melting curves of the samples were determined by RT-qPCR analysis. The data obtained were analyzed using LightCycler480 Instrument Software Version1.5.1.

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

**2.5.1. Housekeeping gene**
B-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'

## 3. RESULTS

### 3.1. 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 perikaryon in most neurons in the CA1region of the hippocampus in the control and sham groups. (Fig. 3-a). 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 IR group showed morphological changes consistent with degeneration. Most of the neurons of the IR group showed dark staining because of the condensation of their cytoplasm and nucleus, which is an indicator of degeneration. The amount of CA1 neurons reduced significantly in the IR group. Because the distance between cells increased, the neurons were spaced apart and randomly distributed and showed an irregular order (Fig. 3-b).

It was determined that hippocampal neuron damage decreased, and a statistically significant increase in the number of neurons was observed in the Pol 60 group compared to the IR group. There was no significant difference in neuronal damage and amount of neuron in Pol 30 group (Fig. 3-c and 3-d).

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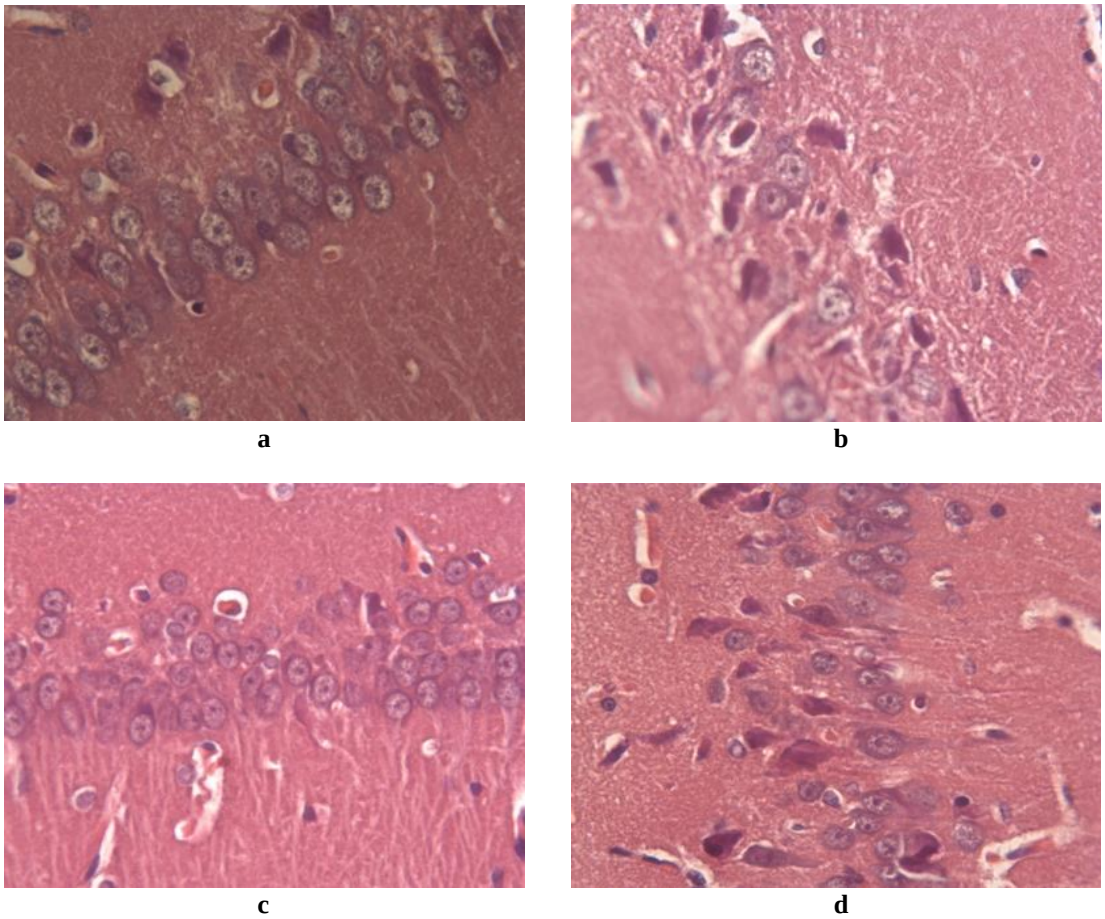

**a** **b**

**c** **d**

**Figure 3.** Hippocampal CA1 region in the control and experimental groups, H&E, 40X. **a.** Control group (staining of neurons are homogeneous, large and round-shaped nuclei, prominent nucleoli) , **b.** IR group (dark and shrunken neurons with many pycnotic nuclei, a large distance between neurons due to loss of neurons, irregularly distributed neurons), **c.** Pol 60 group (Only a few damaged neurons were observed). **d.** Pol 30 group (Neurons with multiple hyperchromatic and pycnotic nuclei and also neuron loss was observed).

### 3.2. Biochemical Analysis Results

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

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

| PARAMETER* | Control (n=8) | Sham (n=8) | IR (n=8) | Pol 30 (n=8) | Pol 60 (n=8) |
|---|---|---|---|---|---|

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| | | | | | |
|---|---|---|---|---|---|
| CAT (U/mg.protein) | 0.98±0.32[a] | 0.81±0.27[a] | 0.93±0.64[a] | 1.09±0,74[b] | 1.12±0,79[b] |
| SOD (U/mg.protein) | 5.53±0.72[a] | 5.43±0.25[a] | 4.44±1.08[b] | 5.39±0,95[a] | 5.88±0,45[a] |
| MDA (nmol/mg.protein) | 9.34±0.2[a] | 10.31±0.6[a] | 17.32±0.1[b] | 14.85±0.7[c] | 11.15±0.5[d] |
| T.Protein (mg /ml) | 88.5±0.2 | 91.3±0.8 | 90.8±0.7 | 78.5±0.6 | 81.3±0.4 |

Results are given as mean ± SD.
* : 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).

There was no significant difference between the control and sham groups in the CAT values. A slight decrease was determined in the IR group in the CAT levels compared to the control, but this value was not significant. The CAT values of Pol 30 and Pol 60 groups increased significantly when compared with control and IR groups. There were no statistically significant differences between the Pol 30 and Pol 60 groups (Fig. 4).

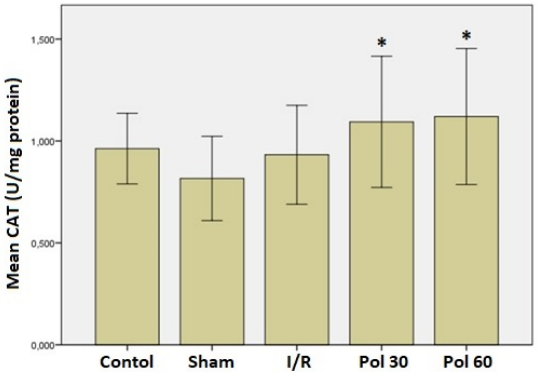


**Figure 4.** Average CAT levels in brain tissues. (*compared to control p < 0.05)

There was no significant difference between the SOD values of the control and sham groups. A significant reduction was detected in the SOD values of the IR group compared to the control. Compared to the IR group, the SOD values of the Pol 30 and Pol 60 groups increased significantly. No significant difference was exhibited between Pol 30 and Pol 60 groups in terms of the SOD values (Fig. 5).

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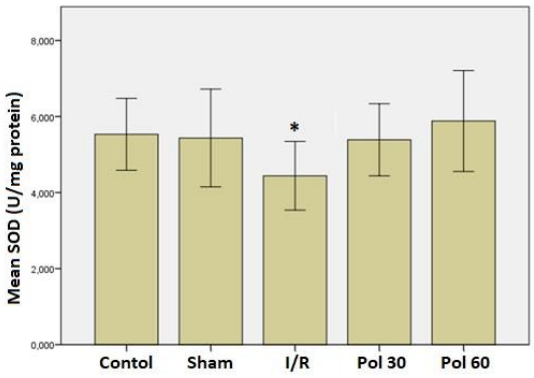


**Figure 5.** Average SOD levels in brain tissues. (*compared to control $p < 0.05$)

The mean MDA value increased statistically significantly in the IR group compared to the control. The MDA levels of the Pol 30 and Pol 60 groups were significantly decreased when compared to the IR. The decrease in the MDA value of the Pol 60 group was closer to the control group. There was also a statistically significant difference between Pol 30 and Pol 60 groups (Fig. 6).

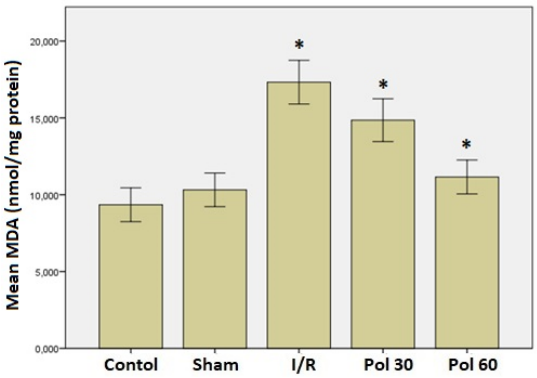


**Figure 6.** Average MDA levels in brain tissues. (*compared to control $p < 0.05$)

There were no significant differences between the control, sham, and IR groups in total protein values. A significant decrease was observed in the total protein values of the Pol 30 and Pol 60 groups compared to the IR group. There were no significant differences between the Pol 30 and Pol 60 groups in terms of the total protein values.

### 3.3. Molecular Analysis Results

TNF-α mRNA expression levels of the groups are given in Figure 7. RNA was isolated from brain tissues and TNF-α mRNA expression level was determined by RT-qPCR. According to the results, there were no differences between the control group and the sham group in mRNA expression values

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in brain tissue. TNF-α mRNA expression levels significantly increased in the IR group, which was not given polydatin by applying only ischemia/reperfusion, compared to control and sham groups.

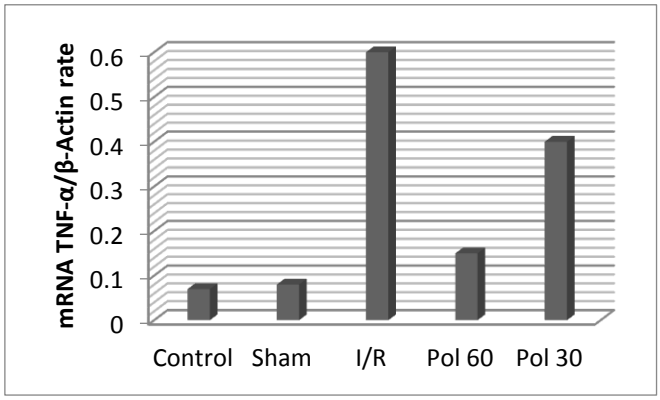


**Figure 7.** TNF-α mRNA expression levels of the experimental groups.

A significant reduction was observed in the mRNA expression level in the Pol 60 group, in which 60 mg/kg Polydatin was administered before ischemia-reperfusion as a preservative compared to the IR group, and it was determined that it reduced to a value close to the control and sham groups. In the Pol 30 group, the TNF-α mRNA expression value slightly decreased compared to the IR group, but this reduction was not significant.

## 4. DISCUSSION

In the first few minutes of ischemia, a cytotoxic response occurs, including oxidative stress, proinflammatory response, neuronal death, and neurological injury [47-49]. Therefore, inhibition of the inflammatory response and activation of the antioxidant defense system in the early phase of ischemia are important for reducing ischemic damage [14]. For this reason, this study, aims to examine the protective effects of polydatin, which has a potential antioxidant effect as in many polyphenol compounds, against cerebral ischemia/reperfusion injury. Various animal models of cerebral ischemia have been improved to illustrate the clinical condition of humans as exactly as possible. The anatomy of cerebral vasculature does not differ greatly in rodent and humans, so rat models have often been used in animal experiments on brain ischemia [19].

MDA, SOD, CAT and total protein values are important markers for oxidative stress. MDA is formed as a result of lipid peroxidation and is an important organic compound. It has been shown in previous studies that MDA levels increase significantly after ischemia [50]. In the study of Li et al. [33], in the four – vessels occlusion model of cerebral ischemia, it was reported that the MDA level increased 3-fold. CAT and SOD are antioxidant enzymes. They are protective against the detrimental effects of super oxide radicals. The activity of this enzyme decreases in cerebral ischemia [50]. Hippocampal CAT and SOD levels decreased by 32% and 27%, respectively, in rats that were applied the four-vessel occlusion model [33].

In this study, it was detected that while MDA level rose, SOD and CAT levels fell, and there was no significant change in the total protein level in the IR group rats compared to the control group. It was observed that MDA level reduced significantly in Pol 30 and Pol 60 groups, in which 30 and 60 mg/kg polydatin were administered as a protective. Especially the decrease in the Pol 60 group was closer to the control. The SOD and CAT values of these groups were significantly higher compared to the I / R group. The mean total protein level decreased significantly in the Pol 30 and Pol 60 groups compared to the control and IR groups.

Previous studies support the data obtained in our study. Li et al. [33] applied polydatin to rats for 30 days after cerebral ischemia induced by the four-vessel occlusion method and found that the MDA level decreased by 40%. Also, this decrease varied depending on the concentration. It was observed that polydatin administered at doses of 25mg/kg and 50mg/kg decreased the MDA levels by 49% and 57%, respectively [33]. In addition, in the same study, it was observed that the application of polydatin increased CAT and SOD activities approximately 1,4 times [33]. Ji et al. [14] applied 50 mg/kg polydatin to rats immediately after ischemia created by the cerebral artery occlusion model and found that the levels of SOD1 protein and mRNA expression increased 2,3 times. In another study, it was determined that cell viability increased significantly, MDA level decreased and SOD activity increased with polydatin administration against ischemic damage due to oxygen-glucose deprivation [23]. Similar results were also obtained in a study on the effects of polydatin on learning and memory [33].

These results demonstrate that polydatin can develop cellular antioxidant properties and effectively decrease neuronal cell loss and damaged area to reduce oxidative stress caused by cerebral ischemia. However, it can be said that the protective effect of polydatin against ischemia/reperfusion damage is related to its anti-oxidative activity.

After cerebral ischemia reperfusion, degeneration and tissue losses are observed in neurons because of lack of oxygen to the brain tissue. According to the results of the study of Yaidikar et al. [51], H&E-stained sections of the brain tissues of the group in which ischemia was created with the cerebral artery occlusion model showed many degenerated neurons and morphological changes due to edema. In another study, 10 days after ischemia/reperfusion, cresyl violet staining was performed to the brain tissues. In the brain tissues, concentrated, pyknotic and shrunken neurons were determined in the CA 1 region of the hippocampus [52]. In another study, Janyou et al. [53] observed that neurons in the cortex region of the brains, which were removed 24 hours after ischemia/reperfusion, were composed of a pycnotic nucleus and a vacuole surrounding it. In the study of Wang et al. [54], necrosis, reticular lesions, pycnotic or lost nuclei in neurons, cytoplasmic dissolution, and cellular edema were observed in the brain tissue of mice induced with cerebral ischemia/reperfusion.

The results of our study are also similar to previous studies. In the IR group, hippocampal CA1 neurons showed morphological changes consistent with degeneration. Dark staining and loss of CA 1 neurons were detected in the IR group because of the densification of the cytoplasm and nucleus of the neurons. In addition, the neurons showed a spaced and random order due to the increased intercellular distance. Neuronal injury in the hippocampus decreased and the number of neurons increased statistically significantly, especially in the Pol 60 group compared to the IR group. These

findings show that polydatin, a powerful antioxidant, neutralizes free radicals caused by ischemia/reperfusion, prevents neuronal loss in the brain, and thus prevents the brain from being damaged.

In ischemia, some neuronal cells (microglia cells, neurons, astrocytes, endothelial cells) that secrete inflammatory cytokines are also activated. In this study, we determined the mRNA expression amounts of the TNF-α (tumor necrosis factor) gene, which is one of the inflammation factors, is released from endothelial cells due to the increase of superoxide radicals as a result of ischemia and reperfusion. The results of this study exhibited that the mRNA expression level of the TNF-α gene was significantly higher in the IR group compared to the control. In the Pol 60 group, the mRNA expression level rose to a value close to the control.

Similar results are seen in other studies. In many studies, an increase in TNF-α mRNA expression was reported after cerebral ischemia/reperfusion [28,55,56]. Liu et al. [26] found that polydatin statistically significant decreased the high level of TNF-α and IL - 1β caused by ischemia/reperfusion in the kidneys. On the other hand, Xu et al. [18] found that TNF-α, IL - 1β and IL - 6 genes, which rose in serum as a result of oxidative stress after liver and brain injury, decreased with polydatin administration. Li et al. [27] observed that polydatin treatment decreased the amount of mRNA expression of the TNF-α gene, which increased as a result of lung damage. These data show that the effects of polydatin on ameliorating cerebral ischemia/reperfusion damage are achieved by inhibiting TNF-α proinflammatory cytokines.

There are limitations in this study, such as the fact that there is only one drug group and the mechanism of action of the drug used is not clear. In the study, the effects of two different doses of a single drug (polydatin) were compared. Results can be supported by comparison with another drug with proven efficacy. In addition, the protective effect of polydatin against ischemia/reperfusion damage was determined in the study, but the mechanism of action was not studied. Obtained results can be strengthened with immunohistochemical data. Studies on this subject are needed.

## 5. CONCLUSION

According to the findings of this research, the increase in MDA, decrease in SOD and CAT values, increase in TNF-α gene mRNA expression and histological damage in the brain because of cerebral ischemia/reperfusion in rats were restored to normal levels with 30 and 60 mg/kg polydatin administration as protective before ischemia.

As a result, with the data of this study, it has been detected that especially high-dose polydatin supplementation of 60 mg/kg has a neuroprotective effect against oxidative stress damage caused by cerebral ischemia/reperfusion thanks to its antioxidant potential.

Existing risks can be avoided by using polydatin prophylactically before the ischemia-reperfusion injury occurs in humans. For this purpose, dietary habits can be regulated with foods containing polydatin. It may be possible to reduce thrombolytic risks when supported by an exercise program too.

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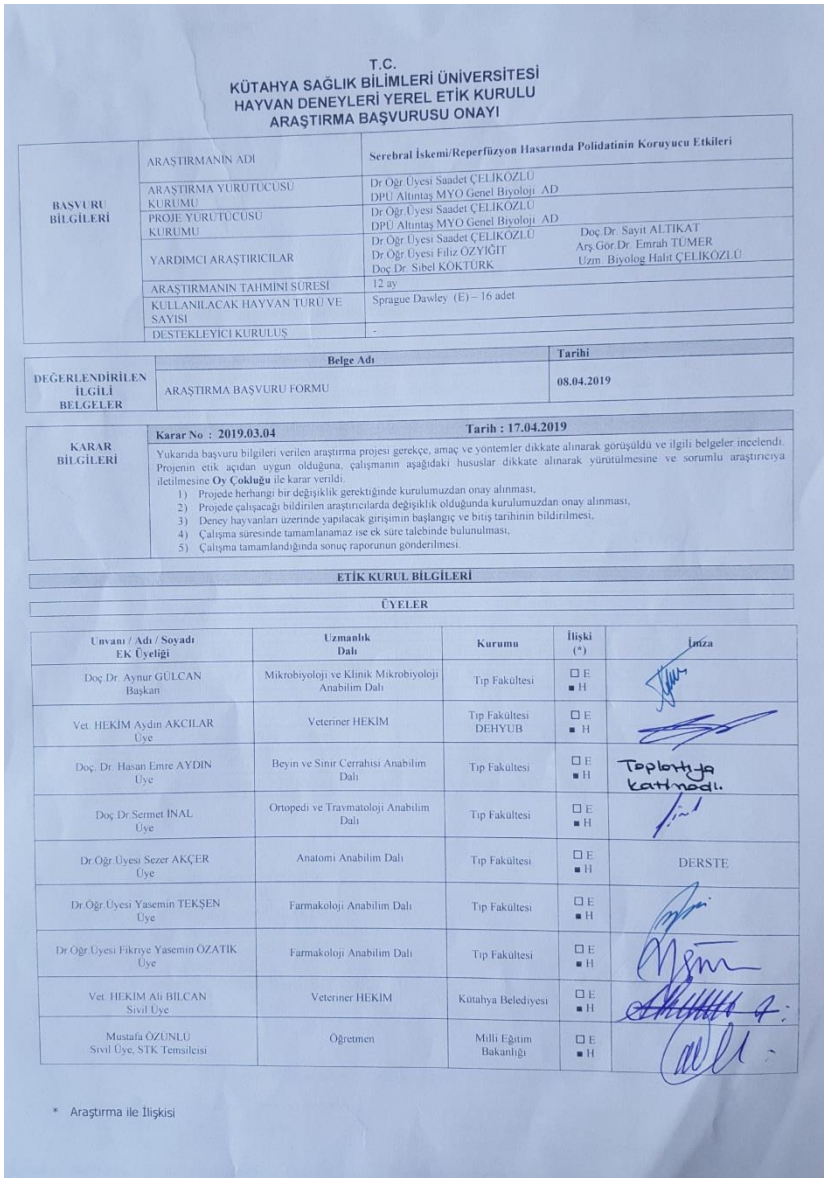
## APPENDIX



T.C.
KÜTAHYA SAĞLIK BİLİMLERİ ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
ARAŞTIRMA BAŞVURUSU ONAYI

| | | |
|---|---|---|
| BAŞVURU BİLGİLERİ | ARAŞTIRMANIN ADI | Serebral İskemi/Reperfüzyon Hasarında Polidatinin Koruyucu Etkileri |
| | ARAŞTIRMA YÜRÜTÜCÜSÜ KURUMU | Dr.Öğr.Üyesi Saadet ÇELİKÖZLÜ<br>DPU Altıntaş MYO Genel Biyoloji AD |
| | PROJE YÜRÜTÜCÜSÜ KURUMU | Dr.Öğr.Üyesi Saadet ÇELİKÖZLÜ<br>DPU Altıntaş MYO Genel Biyoloji AD |
| | YARDIMCI ARAŞTIRICILAR | Dr.Öğr.Üyesi Saadet ÇELİKÖZLÜ<br>Dr.Öğr.Üyesi Filiz ÖZYİĞİT<br>Doç.Dr. Sibel KOKTÜRK<br>Doç.Dr. Sayit ALTIKAT<br>Arş.Gör.Dr. Emrah TÜMER<br>Uzm. Biyolog Halit ÇELİKÖZLÜ |
| | ARAŞTIRMANIN TAHMİNİ SÜRESİ | 12 ay |
| | KULLANILACAK HAYVAN TÜRÜ VE SAYISI | Sprague Dawley (E) – 16 adet |
| | DESTEKLEYİCİ KURULUŞ | - |

| DEĞERLENDİRİLEN İLGİLİ BELGELER | Belge Adı | Tarihi |
|---|---|---|
| | ARAŞTIRMA BAŞVURU FORMU | 08.04.2019 |

**KARAR BİLGİLERİ**

**Karar No : 2019.03.04** **Tarih : 17.04.2019**

Yukarıda başvuru bilgileri verilen araştırma projesi gerekçe, amaç ve yöntemler dikkate alınarak görüşüldü ve ilgili belgeler incelendi. Projenin etik açıdan uygun olduğuna, çalışmanın aşağıdaki hususlar dikkate alınarak yürütülmesine ve sorumlu araştırıcıya iletilmesine **Oy Çokluğu** ile karar verildi.

1) Projede herhangi bir değişiklik gerektiğinde kurulumuzdan onay alınması,
2) Projede çalışacağı bildirilen araştırıcılarda değişiklik olduğunda kurulumuzdan onay alınması,
3) Deney hayvanları üzerinde yapılacak girişimin başlangıç ve bitiş tarihinin bildirilmesi,
4) Çalışma süresinde tamamlanamaz ise ek süre talebinde bulunulması,
5) Çalışma tamamlandığında sonuç raporunun gönderilmesi.

**ETİK KURUL BİLGİLERİ**

**ÜYELER**

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| Doç.Dr. Aynur GÜLCAN Başkan | Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı | Tıp Fakültesi | ☐ E ■ H | |
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| Dr.Öğr.Üyesi Yasemin TEKŞEN Üye | Farmakoloji Anabilim Dalı | Tıp Fakültesi | ☐ E ■ H | |
| Dr.Öğr.Üyesi Fikriye Yasemin ÖZATİK Üye | Farmakoloji Anabilim Dalı | Tıp Fakültesi | ☐ E ■ H | |
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* Araştırma ile İlişkisi