EXPERIMENTAL STUDY

Evaluation of the effects of thymoquinone on red blood cell deformability, morphology, and endothelial nitric oxide synthase (eNOS) synthesis in rat lower extremity ischemia-reperfusion injury

Celalettin Gunay,[1] Hakan Kartal,[1] Ertan Demirdas,[1] Bilgehan Savas Oz,[1] Faruk Metin Comu,[2] Gokhan Erol,[1] Gokhan Arslan,[1] Tayfun Ozdem,[1] Tuna Demirkıran,[1] Muharrem Emre Ozdaş,[3] Isıl Ozdas,[1] Yigit Tokgoz,[1] Veli Can Ozdemir[1]

[1]Department of Cardiovascular Surgery, University of Health Sciences, Gulhane Education and Research Hospital, Ankara-*Türkiye*

[2]Department of Physiology, Kirikkale University Faculty of Medicine, Kirikkale-*Türkiye*

[3]Department of Cardiovascular Surgery, Iğdır Dr. Nevruz Erez State Hospital, Iğdır-*Türkiye*

ABSTRACT

BACKGROUND: Erythrocyte deformability refers to the ability of erythrocytes to bend and twist as they pass through capillaries, which is crucial for tissue perfusion. This study aims to investigate the effects of Thymoquinone treatment on erythrocyte deformability in rats subjected to lower extremity ischemia-reperfusion injury.

METHODS: The study was conducted on Wistar albino rats weighing 400-450 g. The rats were randomly divided into five groups: the control group (C), in which no treatment was applied; the group that received dimethyl sulfoxide (DMSO) as a solvent; the group subjected to 90 minutes of ischemia followed by 90 minutes of reperfusion in the main femoral artery of the lower extremity (IR); the Thymoquinone control group (TQ-C), in which the effects of Thymoquinone alone were examined; and the group that received intraperitoneal Thymoquinone one hour before the ischemia-reperfusion procedure (IR+TQ). At the end of the procedure, intracardiac blood was collected from the rats, and May-Grunwald and Giemsa (MGG) staining, endothelial nitric oxide synthase (eNOS), and erythrocyte deformability indexes were measured.

RESULTS: The study results showed significant differences. Erythrocyte deformability was statistically significantly improved in the group that received Thymoquinone before ischemia-reperfusion compared to the group subjected to ischemia-reperfusion only. Morphological changes in erythrocytes were also statistically significantly better in the IR+TQ group than in the IR group. Immunohistochemical eNOS staining revealed that eNOS activity in the IR group was lower than in the IR+TQ group.

CONCLUSION: Our study demonstrates that Thymoquinone treatment administered before ischemia exerts protective effects against erythrocyte deformation and morphological deterioration by increasing eNOS activity.

Keywords: Erythrocyte deformability; ischemia-reperfusion; endothelial nitric oxide synthase (eNOS); thymoquinone.

Cite this article as: Gunay C, Kartal H, Demirdas E, Oz BS, Comu FM, Erol G, Arslan G, Ozdem T, Demirkıran T, Ozdaş ME, Ozdas I, Tokgoz Y, Ozdemir VC. Evaluation of the effects of thymoquinone on red blood cell deformability, morphology, and endothelial nitric oxide synthase (eNOS) synthesis in rat lower extremity ischemia-reperfusion injury. Ulus Travma Acil Cerrahi Derg 2024;30:715-721.

Address for correspondence: Celalettin Gunay

University of Health Sciences, Gulhane Education and Research Hospital, Ankara, Türkiye

E-mail: celalettingunay@gmail.com

Ulus Travma Acil Cerrahi Derg 2024;30(10):715-721 DOI: 10.14744/tjtes.2024.94055

Submitted: 23.07.2024 Revised: 02.09.2024 Accepted: 27.09.2024 Published: 07.10.2024

INTRODUCTION

The most common mechanism of cellular damage is ischemia, which occurs after the partial or complete interruption of blood flow to a tissue or organ due to various factors, followed by subsequent reperfusion injury.[1] The microvascular circulation is the first system in the body to be affected by reperfusion injury following ischemia.[2] The most essential factor for red blood cells (RBCs) to remain hemodynamically functional is their deformability, which allows them to change shape according to blood flow conditions to minimize resistance to blood flow.[3] Free radicals released into the body after reperfusion injury can react with almost all cellular components. Unsaturated fatty acids found in cell membranes are the most sensitive macromolecules to free radical damage.[4] All changes that occur during reperfusion injury negatively affect the deformability of erythrocytes.[5] The oxidative damage prevention and free oxygen radical scavenging properties of Thymoquinone, derived from Nigella sativa seeds, have been demonstrated in previous in vivo and in vitro studies.[6]

This study aims to investigate, in vivo, the hypothesis that Thymoquinone, with these properties, can correct erythrocyte deformability impairment in lower extremity ischemia-reperfusion injury.

MATERIALS AND METHODS

The study was conducted on December 8, 2023 at the Experimental Animals Laboratory, Ankara, Türkiye, with the approval of the local ethics committee (approval number 696).

Chemicals

Thymoquinone used in the study was obtained from Sigma-Aldrich, without any support from any organization. All materials used in the study were jointly provided by the authors.

Animals

The study was conducted on 30 male Wistar albino rats weighing 400-450 g. The animals were housed for a period of 7 days in standard cages, each containing three rats, with 50% air humidity and a temperature of 21-24°C. A 12-hour automatic light/dark cycle was maintained. All rats had access to standard feed pellets and fresh drinking water throughout the study. The rats were randomly divided into five groups, with six rats in each group. The groups were as follows: Control group (C), Dimethyl Sulfoxide group (DMSO), Ischemia-Reperfusion (IR) group, Thymoquinone control group (TQ-C), and Ischemia-Reperfusion + Thymoquinone (IR+TQ) group.

Technical Procedure

At the beginning of the experimental procedure, the rats were anesthetized with an intramuscular injection of ketamine hydrochloride at a dose of 50 mg/kg and xylazine hydrochloride at 10 mg/kg in the forearm. The procedure was performed in the supine position under a heating lamp. A transverse incision was made in the inguinal region of the lower extremities to expose the main femoral artery and vein. The main femoral vein was cannulated using a 26-gauge venous cannula, and 100 U/kg intravenous heparin was administered to all rats. The same dose of heparin was administered to all rats, regardless of whether they underwent the ischemia-reperfusion procedure, to prevent heparin from affecting the study results. For the ischemia-reperfusion procedure, an atraumatic microvascular clamp was placed on the common femoral artery through a transverse incision in the inguinal region. After a 90-minute ischemic period, the microvascular clamp in the common femoral artery was removed, and reperfusion was allowed for 90 minutes.

Control Group (C, n=6): The rats in this group underwent an inguinal incision, followed by cannulation of the main femoral vein and administration of heparin. Three hours after the procedure, without inducing ischemia, intracardiac blood samples were collected from the rats under anesthesia.

DMSO Group (DMSO, n=6): After the administration of heparin, DMSO, in the same volume as that used to dissolve Thymoquinone (a maximum of 14 mg of Thymoquinone dissolves in 1 mL of DMSO), was administered intraperitoneally to the rats in this group without to examine its effects on erythrocytes. Ischemia was not induced. Heparin was injected through the main femoral vein, and three hours after the procedure, intracardiac blood samples were collected from the rats under anesthesia.

Thymoquinone Control Group (TQ, n=6): The rats in this group were administered 20 mg/kg of Thymoquinone intraperitoneally without inducing ischemia. Heparin was injected through the main femoral vein, and three hours after the procedure, intracardiac blood samples were collected from the rats under anesthesia.

Ischemia-Reperfusion Group (Gr IR, n=6): The rats in this group received heparin injections after cannulation of the main femoral vein following an inguinal incision. An atraumatic microvascular clamp was placed on the main femoral artery, and after 90 minutes of ischemia and 90 minutes of reperfusion, intracardiac blood samples were collected from the rats under anesthesia.

Ischemia-Reperfusion + Thymoquinone Group (IR+TQ, n=6): In this group, after heparin administration through the main femoral vein, Thymoquinone was administered intraperitoneally at a dose of 20 mg/kg one hour before ischemia. An atraumatic microvascular clamp was placed on the main femoral artery, and after 90 minutes of ischemia and 90 minutes of reperfusion, intracardiac blood samples were collected from the rats under anesthesia.[7]

Two peripheral blood smears were prepared from each blood sample. The first peripheral smear was air-dries for May-Grunwald and Giemsa (MGG) staining, then numbered and stored. The second peripheral smear was placed in 98% pure ethanol for 30 minutes for immunohistochemical staining of endothelial nitric oxide synthase (eNOS), then numbered and

preserved. Blood samples taken for deformability evaluation were numbered in heparin-coated blood gas syringes and stored at +4°C.

Deformability Measurements

A constant flow filtrometer system was used to measure the deformability properties of erythrocytes. The system was calibrated within a pressure range of 0.5-4 cmH2O before use. Blood samples taken within 2 hours were analyzed. The heparinized blood was washed three times with phosphate-buffered saline (PBS) buffer, and erythrocyte packs were formed. Subsequently, the erythrocytes were brought to room temperature (25°C) and resuspended in a PBS buffer with a hematocrit (Htc) of 5%. Ten milliliter samples were prepared for analysis. Nuclepore polycarbonate filters (Merck-Whatman) with a 25 mm diameter and 5 µm pore size were used in the system. Separate filters were utilized for each sample. A constant flow rate of 1.5 mL/min. was achieved using an infusion pump (Biopac Systems Inc. - Commat, Ltd.), and the resulting filtration pressure was measured in cmH2O. Actual pressure changes were monitored and recorded by transferring the data to a computer via the Data Acquisition System (Data Acquisition System, Biopac Systems Inc., USA). Two measurements were taken for each sample, and the mean value was used for analysis. Several variables were recorded and analyzed, including the pressure values measured and the time required to reach the measured pressure level.

P L: Erythrocyte Suspension Filtration Pressure

P T: Buffer Solution Filtration Pressure

Relative pressure values were calculated using the following formula:

Rrel: P Erythrocyte / P Buffer.

The relative pressure values provide insight into the deformability of erythrocytes. An increase in this value indicates a negative effect on erythrocyte deformability.[7-8]

Histological Evaluation

Morphological evaluation was performed using May-Grunwald and Giemsa staining of fixed dry blood smear (BS) preparations and observation under a light microscope.[9-10]

Blood smears in the Control (C) group exhibited a normal physiological double concave discoid shape for both red blood cells and white blood cells (WBC). In the Ischemia-Reperfusion (IR) group, shape changes in RBCs, including the presence of echinocytes with round protrusions and teardrop-shaped dacrocytes, were observed.

Histopathological Morphometry

Morphological changes in MGG-stained RBCs and WBCs, as well as changes in echinocytes, teardrop-shaped dacrocytes, and roll formation, were assessed by two blinded researchers using a semi-quantitative scoring system ranging from 0 to 5. The scoring was defined as follows: 1=absent or minimal, 2=mild, 3=moderate, 4=marked, and 5=severe.[9,11-13]

Immunohistochemistry

After deparaffinization and antigen retrieval by indirect immunohistochemistry, sections were placed in TBST (Twin Buffer Solution Buffer, pH 7.4) for 15 minutes and washed with TBST. To prevent non-specific binding, the sections were treated with 1% hydrogen peroxide (Lab Vision, Thermo Scientific, Fremont, USA) for 30 minutes. Following the blocking step, the sections were incubated with the primary antibody eNOS (Boster Bio PA2140-2) diluted at a ratio of 1:100 and applied to the tissue sections. The sections were incubated with the primary antibody overnight at +4°C. Subsequently, the samples were incubated with a biotinylated secondary antibody (Lab Vision, Thermo Scientific) for 20 minutes at room temperature. Streptavidin was then reacted with the enzyme peroxidase for 20 minutes (Lab Vision, Thermo Scientific). The sections were washed with TBST. Finally, the color reaction was enhanced by incubation with a chromogen substrate containing diaminobenzidine (DAB) for approximately 5-10 minutes at room temperature. Contrast staining was performed using Mayer's hematoxylin. Immunoreactivity was evaluated by h-score on the images.[9]

In immunohistochemical (IHC) staining, eNOS expression was moderate in erythrocytes, and it was observed to increase with IR.

H-Score

The semi-quantitative results of immunohistochemical staining were analyzed using the h-score method. Staining intensities were classified as weak (1), moderate (2), and strong (3). Cells were counted in three different microscopic fields for each intensity. The corresponding score was then calculated using the formula: H-score = $\sum$Pi (staining intensity + 1). Pi represents the percentage of labeled cells for each intensity, ranging from 0% to 100%.[9]

Statistical Analysis

The SPSS 25.00 software package (Statical Package for the Social Sciences, Chicago, IL, USA) was used for data analysis. It was found that data distributions did not conform to normality (p=0.01). Additionally, since the number of subjects in each group was n=6, nonparametric tests were applied. The Kruskal-Wallis test was used for statistical analyses, while the Mann-Whitney U test, with correction, was employed to identify the group responsible for the difference. A critical significance level of 0.05 was applied for decision-making.

RESULTS

Deformability Results

It was observed that the deformability measurements differed significantly between the groups. The deformability measurements in the Control, DMSO, and TQ-C groups were significantly lower compared to the IR and IR-TQ groups (p=0.01).

Table 1. Comparison of hemorrhage, edema, vasoconges-tion, inflammation, and Johnsen scores between rat groups

Group	Deformability		p	Difference
	μ	IQR		
C (1)	1.77	0.32	0.01*	1, 2, 4<5<3 (p=0.01)
DMSO (2)	1.91	0.52		
IR (3)	3.09	0.97		
TQ-C (4)	1.94	0.21		
IR-TQ (5)	2.13	0.35		

**Kruskal-Wallis Test. *Significant relationship at the 0.05 level.

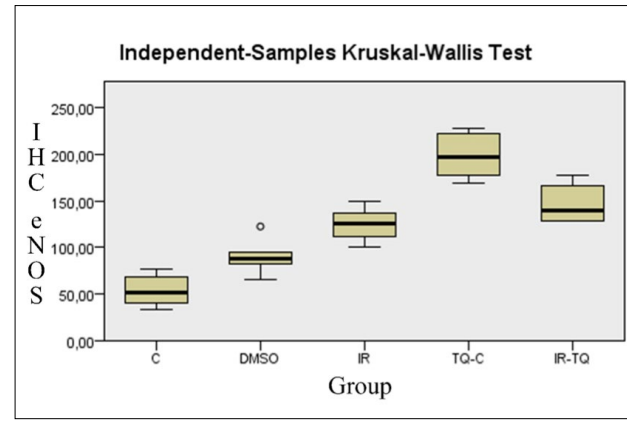

Figure 1. Examination of deformity measurements in groups

Additionally, the measurements in the IR group were higher than those in the IR-TQ group (p=0.01) (Table 1, Fig. 1).

MGG Histochemistry Results

In MGG staining, it was observed that morphologic changes, which were observed very rarely for RBC and WBC in the Control samples, increased significantly with IR (p<0.001). These changes were significantly reduced with TQ application (p<0.05) (Table 2, Figures 2 and 3).

Immunohistochemistry eNOS Results

Immunohistochemical eNOS measurements showed significant differences between the groups. The IHC eNOS measurements in the C and DMSO groups were significantly lower than those in the IR, TQ-C, and IR-TQ groups (p=0.01). Additionally, the measurements in the IR and IR-TQ groups were lower than those in the TQ-C group (p=0.01). (Table 3, Figures 4 and 5).

Table 2. Histopathologic scoring results and statistics for quantitative analysis of morphologic changes in red blood cells (RBC) and white blood cells (WBC) in blood smears (May-Grunwald and Giemsa (MGG) histochemistry staining)

Group	MGG Histochemistry		p	Difference
	μ	IQR		
C (1)	1.86	0.93		1, 2, 3<5<4 (p=0.01)
DMSO (2)	2.35	1.32		
IR (3)	2.45	0.94	0.01*	
TQ-C (4)	4.33	0.88		
IR-TQ (5)	2.92	1.11		

**Kruskal-Wallis Test. *Significant relationship at the 0.05 level.

DISCUSSION

We investigated the protective effects of Thymoquinone against deformability and structural defects in erythrocytes during ischemia-reperfusion injury, and we observed various positive results. The findings provide compelling evidence for the protective role of Thymoquinone, paving the way for further research and potential clinical applications.

Thymoquinone has previously been shown to have cardioprotective, neuroprotective, and antioxidant properties in other studies. (14-16) However, its effects on RBCs, the most important component of microvascular circulation, have not been fully explored.

Our study revealed that erythrocyte deformability was severely impaired following IR, and the erythrocytes were both morphologically and structurally disrupted.

This indicates that reperfusion injury causes similar damage to red blood cells as it does to many solid organs, such as the lungs, kidneys, and brain. (17-20)

Our results align with previous studies, which demonstrated that erythrocyte deformability is impaired in ischemia-reperfusion injury. (21-22)

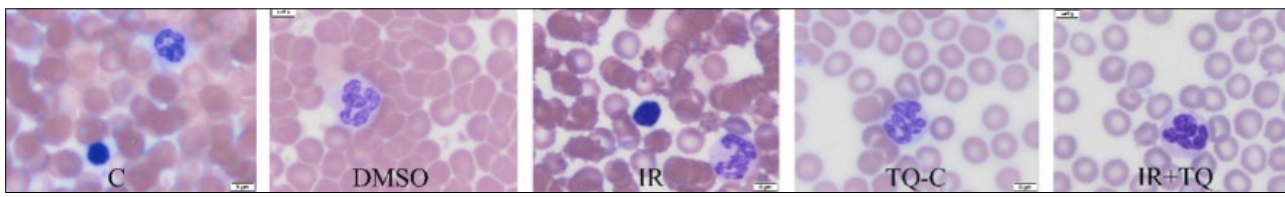

Figure 2. Morphologic changes for RBC and WBC in blood smear by MGG histochemistry staining. (X1000)

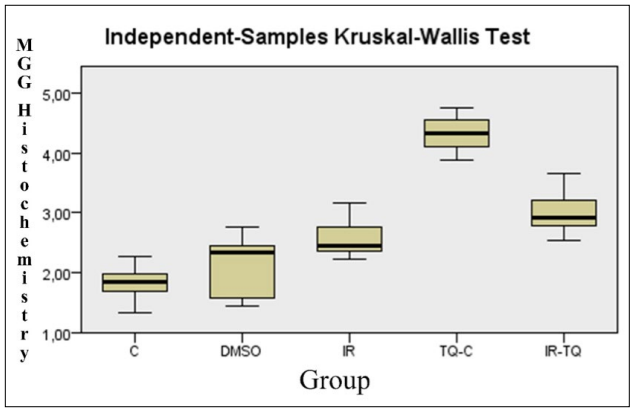

Figure 3. Histopathologic scoring graph for quantitative analysis of morphologic changes for RBC and WBC in blood smear by MGG histochemistry staining.

Table 3. H-score results and statistics of red blood cell (RBC) alterations in immunohistochemistry (IHC) endothelial nitric oxide synthase (eNOS) labeling samples

Group	IHC eNOS		p	Difference
	μ	IQR		
C (1)	50.89	42.66		
DMSO (2)	88.01	56.94		1, 2<3<5<4
IR (3)	126.07	49.26	0.01*	
TQ-C (4)	197.01	58.69		(p=0.01)
IR-TQ (5)	139.00	49.20		

**Kruskal-Wallis Test. *Significant relationship at the 0.05 level.

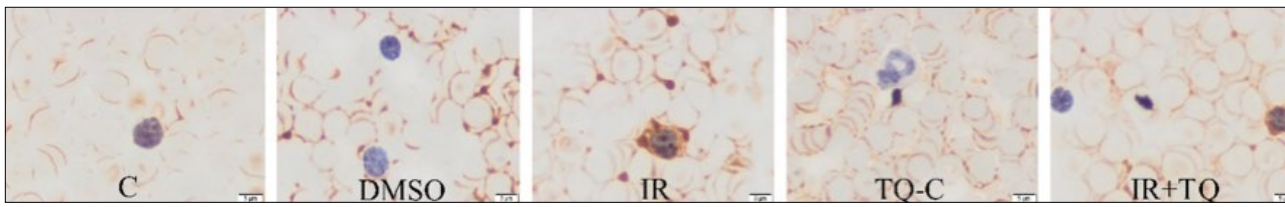

Figure 4. IHC eNOS labeling and changes in RBCs and WBCs in blood smear. (X1000)

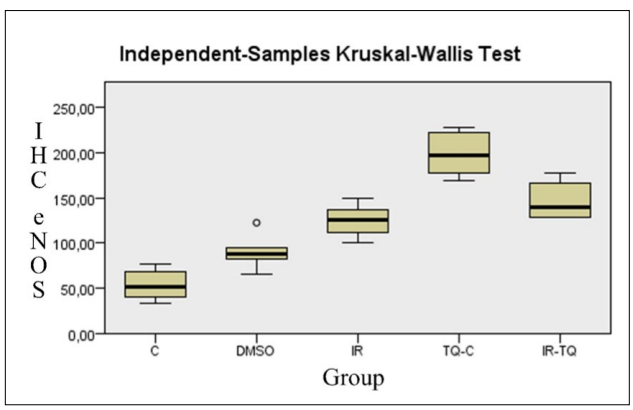

Figure 5. h-score results and statistics of RBC alterations in IHC eNOS labeling samples.

This deformation of erythrocytes, the most critical component of the microcirculation, may result in widespread effects throughout the body.

However, the administration of Thymoquinone prior to ischemia prevented both deformability and structural deterioration in RBCs. Thus, it is suggested that Thymoquinone may have protective effects on the microcirculation by preventing RBC deformation, safeguarding against distant organ damage. The exact pathway through which this deformability-preserving effect occurs is still unknown.

Current evidence suggests that nitric oxide (NO) produced by endothelial cells via eNOS in the vessel wall, is a key regulator of blood flow and blood pressure. In addition to endothelial cells, RBCs also possess catalytically active eNOS, although its role is controversial and poorly understood. (13) Increased activation of RBC NO synthase is thought to reduce RBC deformability. (23)

Moreover, NO also causes capillary vasodilation, as demonstrated in previous studies. (24) In our study, the eNOS activity in the IR+TQ group was higher than that in the IR group. Based on these findings, it is suggested that the administration of Thymoquinone during reperfusion injury may preserve the morphology and deformability of RBCs by increasing eNOS activity in RBCs. The fact that erythrocytic eNOS activity was higher in the TQ-C group, which received only Thymoquinone without ischemia, compared to the other groups, further supports the idea that Thymoquinone increases eNOS activity.

While it is unlikely that this is the only mechanism by which Thymoquinone protects RBC structures during reperfusion injury, it can be argued that it is one of the key elements in its mechanism of action.

CONCLUSION

Our study results indicate that Thymoquinone may increase NO production on RBCs and may protect RBCs in ischemia-reperfusion injury. The findings are promising for the potential clinical application of Thymoquinone's RBC-protective properties. However, several important questions remain

unanswered. Determining the optimal dosage and timing of administration is crucial to maximize efficacy and minimize possible side effects. Further investigation into the safety and efficacy of Thymoquinone is needed in larger clinical trials before widespread clinical adoption.

Limitations

The statistical analysis indicated that a larger sample size of animals would improve the statistical evaluation. It is suggested that these statistical limitations can be addressed by increasing the number of animals in future studies.

Ethics Committee Approval: This study was approved by the Kobay Experimental Animals Laboratory Ethics Committee (Date: 24.11.2023, Decision No: 696).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: C.G., B.S.Ö.; Design: H.K., E.D.; Supervision: G.E., H.K.; Resource: F.M.C., H.K.; Materials: G.A.; Data collection and/or processing: T.Ö., C.G.; Analysis and/or interpretation: T.D.; Literature search: M.E.Ö., I.Ö.; Writing: Y.T., V.C.Ö.; Critical reviews: H.K., C.G.

Conflict of Interest: None declared.

Financial Disclosure: The author declared that this study has received no financial support.

REFERENCES

1. Tunç E, Durgun V, Akıncı O, Ergün S, Şimşek O, Bolayırlı IM, et al. The role of geraniol on hepatic ischemia-reperfusion injury model in rats. Ulus Travma Acil Cerrahi Derg 2024;30:390–6. [CrossRef]
2. Arslan M, Comu FM, Isik B, Ozturk L, Kesimci E. Effect of dexmedetomidine on erythrocyte deformability during ischemia-reperfusion injury of liver in diabetic rats. Bratisl Lek Listy 2012;113:687–91. [CrossRef]
3. Barshtein G, Gural A, Arbell D, Barkan R, Livshits L, Pajic-Lijakovic I, et al. Red blood cell deformability is expressed by a set of interrelated membrane proteins. Int J Mol Sci 2023;24:12755. [CrossRef]
4. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 5th edition. Oxford University Press, USA, 2015. [CrossRef]
5. Nemeth N, Lesznyak T, Szokoly M, Furka I, Miko I. Allopurinol prevents erythrocyte deformability impairing but not the hematological alterations after limb ischemia-reperfusion in rats. J Invest Surg 2006;19:47–56.
6. Aydin MS, Kocarslan A, Kocarslan S, Kucuk A, Eser İ, Sezen et al. Thymoquinone protects end organs from abdominal aorta ischemia/reperfusion injury in a rat model. Rev Bras Cir Cardiovasc 2015;30:77–83.
7. Kartal H, Comu FM. Effect of melatonin on erythrocyte deformability in mice with ischemia-reperfusion injury in skeletal muscle. J Surg and Res 2020:3;262–71. [CrossRef]
8. Arslan M, Comu FM, Işık B, Unal Y, Cekmen N, Kurtipek O. Effects of a general anaesthetic agent, propofol, on erythrocyte deformability. Bratisl Med J 2010;111:126–8.
9. Široká M, Franco C, Guľašová Z, Hertelyová Z, Tomečková V, Rodella LF, et al. Nuclear factor-kB and nitric oxide synthases in red blood cells: good or bad in obesity? A preliminary study. Eur J Histochem 2020;64:3081. [CrossRef]
10. Kleinbongard P, Schulz R, Rassaf T, Lauer T, Dejam A, Jax T, et al. Red blood cells express a functional endothelial nitric oxide synthase. Blood 2006;107:2943–51. [CrossRef]
11. Ozer A, Comu FM, Kucuk A, Kilic Y, Alkan M, Oktar L, et al. L. Effects of dexmedetomidine and thymoquinone on erythrocyte deformability in lower limb ischemia reperfusion injury in streptozotocin-induced diabetic rats. Bratisl Lek Listy 2018;119:642–5. [CrossRef]
12. Erol G, Kartal H, Comu FM, Cetin E, Demirdas E, Sicim H, et al. Effects of N-Acetylcysteine and N-Acetylcysteine amide on erythrocyte deformability and oxidative stress in a rat model of lower extremity ischemia-reperfusion injury. Cardiol Res Pract 2020;2020:6841835. [CrossRef]
13. Leo F, Suvorava T, Heuser SK, Li J, LoBue A, Barbarino F, et al. Red blood cell and endothelial enos independently regulate circulating nitric oxide metabolites and blood pressure. Circulation 2021;144:870–89.
14. Yardımcı M, Göz M, Aydın MS, Kankılıç N, Temiz E. Antioxidant actions of thymoquinone, silymarin, and curcumin on experimental aortic ischemia-reperfusion model in wistar albino rats. Braz J Cardiovasc Surg 2022;37:807–13. [CrossRef]
15. Gökce EC, Kahveci R, Gökce A, Cemil B, Aksoy N, Sargon MF, et al. Neuroprotective effects of thymoquinone against spinal cord ischemia-reperfusion injury by attenuation of inflammation, oxidative stress, and apoptosis. J Neurosurg Spine 2016;24:949–59. [CrossRef]
16. Xiao J, Ke ZP, Shi Y, Zeng Q, Cao Z. The cardioprotective effect of thymoquinone on ischemia-reperfusion injury in isolated rat heart via regulation of apoptosis and autophagy. J Cell Biochem 2018;119:7212–7.
17. Kartal S, Şen A, Tümkaya L, Erdivanlı B, Mercantepe T, Yılmaz A. The effect of dexmedetomidine on liver injury secondary to lower extremity ischemia-reperfusion in a diabetic rat model. Clin Exp Hypertens 2021;43:677–82. [CrossRef]
18. Hsu KY, Chen CH, Shih PC, Huang CJ. Adverse effects of bilateral lower limb ischemia-reperfusion on inducing kidney injuries in rats could be ameliorated by platonin. Acta Anaesthesiol Taiwan 2012;50:63–8.
19. Küçükebe ÖB, Özzeybek D, Abdullayev R, Ustaoğlu A, Tekmen I, Küme T. Effect of dexmedetomidine on acute lung injury in experimental ischemia-reperfusion model. Braz J Anesthesiol 2017;67:139–46. [CrossRef]
20. Karabag H, Koçarslan S. Comparison of the effects of thymoquinone and silymarin on the brain of rats having ischemia-reperfusion in the lower extremities. Ann Ital Chir 2020;91:131–6.
21. Grau M, Kollikowski A, Bloch W. Remote ischemia preconditioning increases red blood cell deformability through red blood cell-nitric oxide synthase activation. Clin Hemorheol Microcirc 2016;63:185–97. [CrossRef]
22. Semigran MJ, Cockrill BA, Kacmarek R, Thompson BT, Zapol WM, Dec GW, et al. Hemodynamic effects of inhaled nitric oxide in heart failure. J Am Coll Cardiol 1994;24:982–8. [CrossRef]

DENEYSEL ÇALIŞMA - *ÖZ*

Sıçan alt ekstremite iskemi-reperfüzyon hasarında timokinonun kırmızı kan hücresi deforme edilebilirliği, morfolojisi ve eNOS sentezi üzerine etkilerinin değerlendirilmesi

Celalettin Günay,[1] Hakan Kartal,[1] Ertan Demirdaş,[1] Bilgehan Savaş Oz,[1] Faruk Metin Çomu,[2] Gökhan Erol,[1] Gökhan Arslan,[1] Tayfun Özdem,[1] Tuna Demirkıran,[1] Muharrem Emre Özdaş,[3] Işıl Özdaş,[1] Yiğit Tokgoz,[1] Veli Can Özdemir[1]

[1]Sağlık Bilimleri Üniversitesi. Gülhane Eğitim ve Araştırma Hastanesi, Kalp ve Damar Cerrahisi Anabilim Dalı, Ankara, Türkiye

[2]Kırıkkale Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, Kırıkkale, Türkiye

[3]Iğdır Dr. Nevruz Erez Devlet Hastanesi, Kalp ve Damar Cerrahisi Bölümü, Iğdır, Türkiye

AMAÇ: Eritrosit deformabilitesi eritrositlerin kapiller damarlardan geçerken yaptıkları eğilip bükülme hareketidir ve doku perfüzyonu için hayati öneme sahiptir. Çalışmamızda alt ekstremite iskemi-reperfüzyon uygulanan ratlarda Thymoquinone tedavisinin eritrosit deformabilitesini nasıl etkilediği araştırılmıştır.

GEREÇ VE YÖNTEM: Çalışmamızda ağırlıkları 400-450 gr arasında değişen wistar albino ratlar kullanıldı. Ratlar rastgele 5 gruba ayırıldı. Herhangi bir işlem uygulanmayan kontrol grubu (C), yalnızca çözücü madde DMSO verilen grup (DMSO), alt ekstremie ana femoral arterden 90 dakika iskemi ve 90 dakika reperfüzyon uygulanan grup (IR), Thymoquinone'un tek başına etkilerinin incelendiği Thymoquinone control grubu (TQ-C) ve iskemi reperfüzyon prosedüründen 1 saat önce intraperitoneal Thymoquinone verilen grup (IR+TQ). Prosedür bitiminde ratlrdan intrakardiak kan alınarak May-Grunwald ve Giemsa (MGG), endotelial nitrik oksit sentaz (eNOS) ve eritrosit deformabilite indexleri çalışıldı.

BULGULAR: Çalışma sonuçlarında anlamlı değişikliklere rastlanıldı. İskemi reperfüzyon öncesi Thymoquinone verilen grupta eritrosit deformabilitesi sadece iskemi reperfüzyon yapılan gruba göre istatiksel anlamlı olarak daha iyi tespit edildi. Eritrositlerin morfolojik değişiklikleri de IR+TQ grubunda IR grubuna göre istatiksel anlamlı olarak daha iyi bulundu. İmmünohistokimyasak eNOS boyamasında ise IR grubundaki eNOS aktivitesinin IR+TQ grubuna göre daha düşük düzeyde olduğu tespit edildi.

SONUÇ: Çalışma sonuçlarımızda iskemi öncesi uygulanan Thymoquinone tedavisinin eNOS aktivitesini arttırarak eritrosit deformasyonunu ve eritrositlerin morfolojik bozulmalarına karşı koruyucu özelliğinin olduğunu gösterdi.

Anahtar sözcükler: eNOS; eritrosit deformabilite; iskemi-reperfüzyon; timokinon.

Ulus Travma Acil Cerrahi Derg 2024;30(10):715-721 DOI: 10.14744/tjtes.2024.94055