EXPERIMENTAL STUDY

Evaluation of the effects of rosmarinic acid on red blood cell deformability, morphology, and nitric oxide in rat lower limb skeletal muscle ischemia-reperfusion injury

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ABSTRACT

BACKGROUND: Erythrocyte deformability, the ability of red blood cells to bend and twist as they pass through capillaries, is essential for tissue perfusion. This study investigates the effects and underlying mechanisms of rosmarinic acid treatment on erythrocyte deformability in rats subjected to lower limb 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 with no treatment (C), the group receiving only the solvent dimethyl sulfoxide (DMSO), the ischemia-reperfusion group (IR) subjected to 90 minutes of ischemia followed by 90 minutes of reperfusion in the femoral artery of the lower limb, the rosmarinic acid control group (RA-C) to assess the effects of rosmarinic acid alone, and the group (IR+RA) in which rosmarinic acid was administered intraperitoneally one hour before the ischemia-reperfusion procedure. At the end of the experiment, intracardiac blood samples were collected. Analyses included May-Grünwald-Giemsa (MGG) staining, measurement of endothelial nitric oxide synthase (eNOS), erythrocyte deformability indexes, malondialdehyde (MDA), and superoxide dismutase (SOD) levels.

RESULTS: Significant findings were observed in the study. Erythrocyte deformability was statistically significantly improved in the group that received rosmarinic acid prior to ischemia-reperfusion compared to the group that underwent ischemia-reperfusion alone. Morphological changes of erythrocytes were also significantly better in the IR+RA group than in the IR group. Immunohistochemical analysis of eNOS staining revealed that eNOS activity was higher in the IR group compared to the IR+RA group. Malondialdehyde (MDA) levels were significantly elevated in the IR group compared to all other groups. Analysis of superoxide dismutase levels showed that the SOD levels in the IR+RA group were significantly higher than those in the other groups.

CONCLUSION: Our findings indicate that rosmarinic acid treatment administered prior to ischemia provides protective effects against erythrocyte deformation and morphological deterioration. It is suggested that the improvement in deformability may be mediated by increased SOD activity, which reduces reactive oxygen anions, by-products of nitric oxide (NO) production, thereby exerting an antioxidant effect and enhancing the beneficial actions of NO.

Keywords: Rosmarinic acid; ischemia-reperfusion; erythrocyte deformability; nitric oxide.

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INTRODUCTION

Erythrocytes (red blood cells, RBCs) must undergo shape changes to pass through capillaries and facilitate gas exchange of oxygen and carbon dioxide in distal tissues. This ability to alter shape, an essential characteristic of erythrocytes, is referred to as deformability.[1]

Red blood cells are the most abundant cells in the body, accounting for approximately 70% of all cells.[2] It has been suggested that redox balance, which affects both the RBC membrane and membrane-associated proteins, is closely associated with RBC deformability.[3] Recent research has focused on the molecular mechanisms of nitric oxide (NO) production, both RBC-derived and non-RBC-derived, which play a critical role in maintaining RBC deformability in the microcirculation. Reducing oxidative reactions that affect RBC structural proteins and increasing NO bioavailability have been suggested as protective strategies for preserving RBC deformability.[4]

Impaired erythrocyte deformability due to oxidative stress following skeletal muscle ischemia and subsequent reperfusion injury has been demonstrated in numerous studies.[5]

Rosmarinic acid (RA) is a compound with a molecular structure containing two phenolic rings and is found in various plants. Originally isolated from rosemary (Rosmarinus officinalis, Lamiaceae) and named after this plant, RA is also present in many other members of the Lamiaceae family, including sage, mint, thyme, lemon balm, basil, oregano, and others.[6-8]

Numerous in vitro studies have been conducted to demonstrate the beneficial effects of RA. Its confirmed activities include antioxidant, antibacterial, antiviral, analgesic, anti-inflammatory, and cytoprotective effects.[9]

In our study, we investigated the effects of rosmarinic acid, known for its significant therapeutic properties, on erythrocyte deformability impaired by ischemia-reperfusion injury, as well as its relationship with nitric oxide.

MATERIALS AND METHODS

This study investigated the effects of ischemia-reperfusion (IR) on rat erythrocytes and the potential protective role of rosmarinic acid. Ethical approval was obtained from the Kobay DHL A.S. LocaL Ethical committee in Ankara, Türkiye (Approval Number: 694, Date: 08.12.2023), and this study was conducted on December 8, 2023 in the Experimental Animals Laboratory.

Chemicals

All materials, including rosmarinic acid (purchased from Sigma-Aldrich), were provided by the authors. No external funding was received for this research.

Animals

Thirty male Wistar albino rats (400-450 g) were housed in standard cages (three rats per cage) for seven days prior to the experiment. Environmental conditions were maintained at 50% humidity, with a temperature range of 21-24°C, under a 12-hour light/dark cycle. Rats had free access to standard chow and fresh water throughout the study. The animals were randomly assigned to five groups (n=6 per group): Control (C), Dimethyl Sulfoxide (DMSO), Ischemia-Reperfusion (IR), Rosmarinic Acid Control (RA-C), and Ischemia-Reperfusion + Rosmarinic Acid (IR+RA).

Experimental Procedure

Rats were anesthetized with intramuscular ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). The surgical procedure was performed under a heating lamp with the rats positioned supine. A transverse incision was made in the inguinal region to expose the main femoral artery and vein. The femoral vein was cannulated with a 26-gauge catheter, and all rats received 100 U/kg of intravenous heparin to prevent clotting. To induce IR, an atraumatic microvascular clamp was applied to the femoral artery for 90 minutes, followed by 90 minutes of reperfusion.

• C group: Received heparin and underwent only an inguinal incision.

• DMSO group: Received heparin, intraperitoneal DMSO (maximum 25 mg rosmarinic acid dissolved in 1 mL DMSO),[10] and an inguinal incision. This group was included to assess the effects of DMSO used as the RA solvent.

• RA-C group: Received heparin, 50 mg/kg intraperitoneal RA,[11] and an inguinal incision.

• IR group: Received heparin, an inguinal incision, and underwent the IR procedure.

• IR+RA group: Received heparin and 50 mg/kg intraperitoneal RA one hour prior to the inguinal incision and IR procedure.[12]

Three hours after the procedure, intracardiac blood samples were collected under anesthesia. Two peripheral blood smears were prepared: one air-dried for May-Grünwald-Giemsa (MGG) staining, and one fixed in ethanol for immunohistochemical staining of endothelial nitric oxide synthase (eNOS). Blood for deformability testing was collected in heparinized syringes and stored at 4°C. Plasma for superoxide dismutase (SOD) and malondialdehyde (MDA) analysis was obtained by centrifugation and stored at -80°C.

Deformability Measurements

Erythrocyte deformability was measured using a constant-flow filtrometer calibrated to a pressure range of 0.5-4 cm-H2O. Within two hours of collection, blood samples were washed with phosphate-buffered saline (PBS), and erythro-

cytes were resuspended in PBS to a hematocrit of 5%. A 10 mL sample was passed through a 25 mm diameter Nucleopore polycarbonate filter (5 µm pore size) at a constant flow rate of 1.5 mL/min using an infusion pump. The resulting filtration pressure (cmH_2O) was recorded using a data acquisition system. Two measurements were taken per sample and averaged. Relative pressure (Rrel) was calculated as the ratio of erythrocyte suspension filtration pressure (PL) to buffer solution filtration pressure (PT). An increased Rrel value indicates reduced erythrocyte deformability.[12,13]

Histological Evaluation

Morphological analysis of erythrocytes was performed on MGG-stained blood smears using light microscopy.[14,15] Control samples exhibited the typical biconcave discoid shape. IR-induced changes included the presence of echinocytes (cells with spiky protrusions) and dacrocytes (teardrop-shaped cells) (Fig. 1).

Histopathological Morphometry

Two blinded researchers assessed morphological changes in erythrocytes and leukocytes, including echinocytes, dacrocytes, and "roll formation," on MGG-stained smears. A semi-quantitative scoring system (0-5) was used: 1=absent/minimal, 2=mild, 3=moderate, 4=marked, 5=severe.[14,16,17]

Immunohistochemistry

Ethanol-fixed blood smears underwent antigen retrieval, washing with Tris-buffered saline containing Tween 20 (TBST), and treatment with hydrogen peroxide to block non-specific binding. Slides were incubated overnight at 4°C with a 1:100 dilution of anti-eNOS primary antibody, followed by application of a biotinylated secondary antibody and streptavidin-peroxidase complex. Diaminobenzidine (DAB) was used as the chromogen, and Mayer's hematoxylin served as the counterstain. eNOS immunoreactivity was evaluated using the H-score. Moderate eNOS expression was observed in erythrocytes, with increased expression noted in the IR group (Fig. 2).[14]

H-Score

The H-score was used for the semi-quantitative analysis of eNOS immunohistochemical staining. Staining intensity was graded as weak (1), moderate (2), or strong (3). The percentage of cells at each intensity level (Pi) was determined in three microscopic fields, and the H-score was calculated using the following formula: H-score = ΣPi (Staining Intensity + 1).[14]

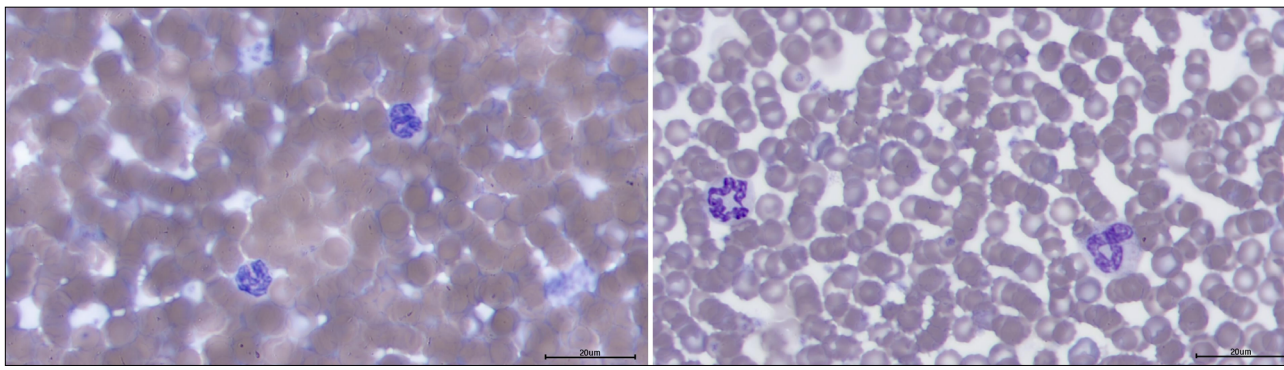

Figure 1. Morphological changes in red blood cells (RBCs) in Control (C) (left) and Ischemia-Reperfusion (IR) (right) samples observed with May-Grünwald-Giemsa (MGG) staining. Echinocytes with protrusions, teardrop-shaped dacrocytes, and roll formations are evident (X1000).

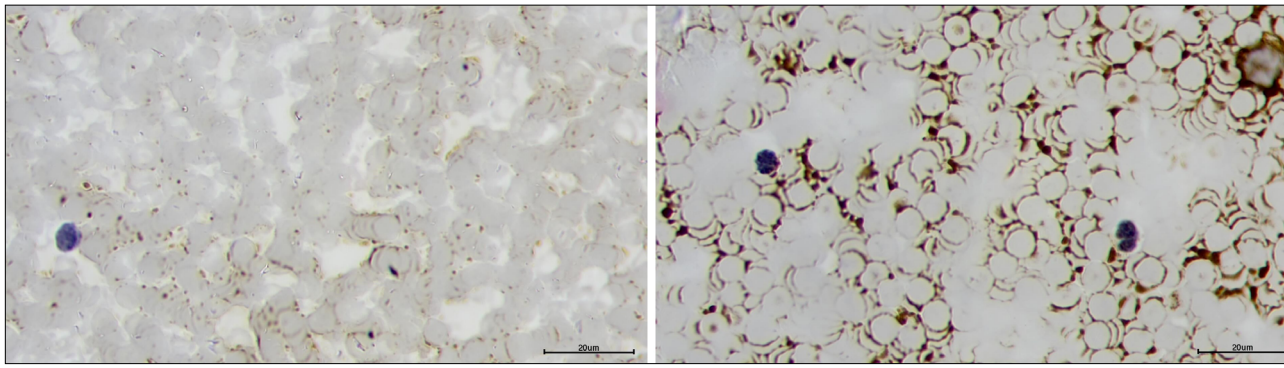

Figure 2. Endothelial nitric oxide synthase (eNOS) staining in red blood cells (RBCs) indicating oxidative stress. A moderate eNOS expression is observed in Control (C) samples (left), while a significantly increased expression is seen in Ischemia-Reperfusion (IR) samples (right) (X1000).

Biochemical Analysis

Enzyme-linked immunosorbent assays (ELISAs) were used to measure plasma levels of SOD and MDA.

SOD (Superoxide Dismutase): SOD levels were measured using a Sunredbio brand ELISA kit (Ref: DZE201110169, Lot: 202311). All procedures were performed according to the manufacturer's instructions. Reagents were brought to room temperature and diluted immediately before use. A stock standard solution was prepared, and SOD standards were serially diluted to concentrations of 64 ng/mL, 32 ng/mL, 16 ng/mL, 8 ng/mL, 4 ng/mL, and 2 ng/mL. Fifty microliters (50 µl) of each standard was added to the appropriate wells. No antibody was added to the standard wells, as the standard solution already contained a biotin-labeled antibody. Samples were added to the wells in a volume of 40 µl. Then, 10 µl of anti-SOD antibody was added to the sample wells. Subsequently, 50 µl of streptavidin-horseradish peroxidase (streptavidin-HRP) was added to both the standard and sample wells. The wells were then coated and incubated at 37°C for 60 minutes. After incubation, the wells were washed five times using an ELISA washer with 350 µl of washing solution. Next, 50 µl of chromogen solution A was added to each well, followed by chromogen solution B. The wells were then covered and incubated in the dark at 37°C for 10 minutes. Finally, 50 µl of stop solution was added to each well, resulting in a color change from blue to yellow. Absorbance was measured at a wavelength of 450 nm within 15 minutes using a microplate reader.

MDA (Malondialdehyde): MDA levels were measured using a Sunredbio brand ELISA kit (Ref: DZE201110157, Lot: 202311). All procedures were conducted in accordance with the manufacturer's instructions. Reagents were brought to room temperature before use and diluted at the time of use. A stock standard solution was prepared using the MDA standard, and serial dilutions were made to obtain final concentrations of 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, and 1.25 ng/mL. Fifty microliters (50 µl) of each standard solution was added to the appropriate wells. No antibody was added to the standard wells, as the standard solution already contained a biotin-labeled antibody. For sample wells, 40 µl of the plasma sample was added, followed by 10 µl of anti-MDA antibody. Then, 50 µl streptavidin-HRP was added to both standard and sample wells. The wells were coated and incubated at 37°C for 60 minutes. Following incubation, the wells were washed five times with 350 µl of washing solution using an ELISA washer. Next, 50 µl of chromogen solution A was added to each well, followed by chromogen solution B. The wells were then covered and incubated in the dark at 37°C for 10 minutes. Finally, 50 µl of stop solution was added to each well, resulting in a color change from blue to yellow. Absorbance was measured at 450 nm using a microplate reader within 15 minutes.

Statistical Analysis

Data analysis was performed using SPSS version 22.00 for Windows (Statistical Package for the Social Sciences, Chicago, IL, USA). Results are presented as mean ± standard deviation (SD). Statistical comparisons were made using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p value ≤0.05 was considered statistically significant.[17]

RESULTS

Deformability Analysis Results

Deformability measurements differed significantly between the groups (p=0.01, p<0.05). The deformability values in the IR group were significantly higher than those in the Control, DMSO, RA-C, and IR+RA groups (p=0.01). Deformability levels were similar among the Control, DMSO, RA-C, and IR+RA groups (p>0.05) (Table 1).

Histological Analysis Results

MGG histochemistry staining scores showed significant differences among the groups (p=0.01, p<0.05). The Control, DMSO, and RA-C groups had significantly lower staining

Table 1. Evaluation of erythrocyte deformability measurements across groups

Group	Deformability		p	Difference
	µ	IQR		
C (1)	1.77	0.32		1, 2, 4, 5 < 3
DMSO (2)	1.91	0.52	0.01*	(p=0.01)
IR (3)	3.09	0.97		
RA-C (4)	2.00	0.52		
IR+RA (5)	2.10	0.58		

**Kruskal-Wallis Test. *Significant difference at p<0.05.

Table 2. Histopathological scoring results and statistical analysis for quantitative evaluation of morphological changes in red blood cells (RBCs) in blood smears using May-Grünwald-Giemsa (MGG) histochemistry staining

Group	MGG Histochemistry Evaluation		p	Difference
	μ	IQR		
C (1)	1.82	1.19		
DMSO (2)	2.28	0.97	0.01*	1, 2, 4 < 5 < 3
IR (3)	4.16	1.63		(p=0.01)
RA-C (4)	2.30	0.96		
IR+RA (5)	3.18	1.06		

**Kruskal-Wallis Test. *Significant difference at p<0.05.

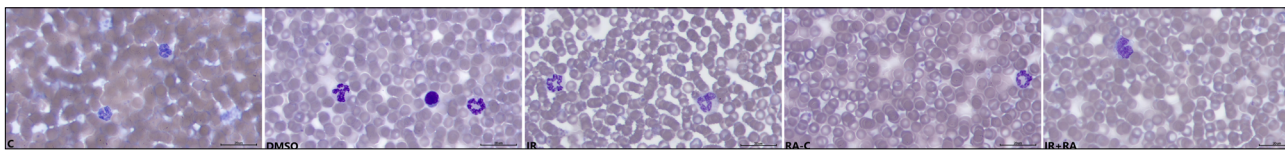

Figure 3. Morphological changes in red blood cells (RBCs) in blood smears stained with May-Grünwald-Giemsa (MGG) histochemistry (X1000). When morphological changes were evaluated in RBCs using MGG histochemistry staining of blood smears, it was observed that membrane protrusions, echinocyte formations, teardrop-shaped dacrocytes, and roll formations were increased in the Ischemia-Reperfusion (IR) group.

scores compared to the IR and IR+RA groups (p=0.01). Additionally, the IR group exhibited higher staining scores than the IR+RA group (p=0.01) (Table 2, Fig. 3).

Immunohistochemical Analysis and eNOS Results

Immunohistochemical (IHC) eNOS staining levels were significantly different among the groups (p=0.01, p<0.05). The IHC eNOS levels in the Control and DMSO groups were significantly lower than those in the IR, RA-C, and IR+RA groups (p=0.01). Furthermore, eNOS levels in the RA-C and IR+RA groups were lower than those in the IR group (p=0.01) (Table 3, Fig. 4).

Biochemical Analysis Results

MDA

MDA levels showed significant differences among the groups (p=0.01, p<0.05). The MDA levels in the IR group were significantly higher than those in all other groups (p=0.01) (Table 4).

SOD

SOD measurements were found to differ significantly between the groups (p=0.01, p<0.05). The difference was attributed to the fact that the SOD levels in the IR+RA group were significantly higher than those in the Control, IR, and RA-C groups (p=0.05) (Table 5).

Table 3. H-score results and statistical analysis of oxidative changes in red blood cells (RBCs) in control (C) samples using immunohistochemical (IHC) labeling of endothelial nitric oxide synthase (eNOS)

Group	eNOS Immunohistochemistry Evaluation		p	Difference
	μ	IQR		
C (1)	91.96	68.02		1, 2 < 4, 5 < 3
DMSO (2)	119.03	68.63	0.01*	(p=0.01)
IR (3)	205.92	85.95		
RA-C (4)	136.87	84.29		
IR+RA (5)	156.18	54.86		

**Kruskal-Wallis Test. *Significant difference at p<0.05.

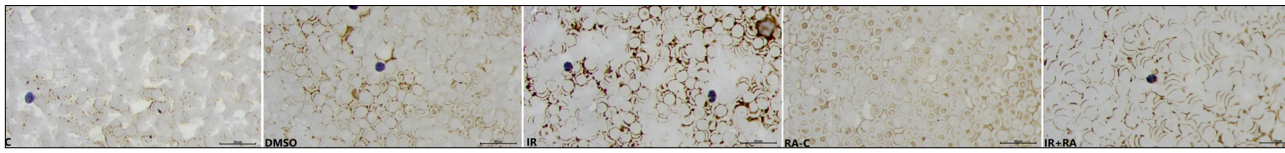

Figure 4. Oxidative changes in red blood cells (RBCs) in blood smears using immunohistochemical (IHC) labeling of endothelial nitric oxide synthase (eNOS) labeling (X1000). When oxidative changes in RBCs in C samples were evaluated using IHC labeling of eNOS, it was observed that eNOS staining intensity was higher in the IR group compared to the other groups.

Table 4. Malondialdehyde (MDA) oxidant levels and statistical analysis across groups

Group	MDA Evaluation		p	Difference
	μ	IQR		
C (1)	14.40	3.13		
DMSO (2)	16.24	0.89	0.01*	3>1, 2, 4, 5
IR (3)	21.73	6.22		(p=0.01)
RA-C (4)	17.35	2.74		
IR+RA (5)	17.42	1.67		

**Kruskal-Wallis Test. *Significant difference at p<0.05.

Table 5. Superoxide dismutase (SOD) levels and statistical analysis across groups

Group	SOD Evaluation		p	Difference
	μ	IQR		
C (1)	3.47	1.26		5 > 1 (p=0.01)
DMSO (2)	7.55	1.63	0.01*	5 > 3 (p=0.04)
IR (3)	5.97	1.74		5 > 4 (p=0.03)
RA-C (4)	6.58	2.66		
IR+RA (5)	10.41	2.98		

**Kruskal-Wallis Test. *Significant difference at p<0.05.

DISCUSSION

In our study investigating the protective effects of rosmarinic acid on red blood cell deformability in lower limb ischemia-reperfusion injury, we obtained several positive findings. The results not only provide strong evidence for the RBC-protective role of rosmarinic acid but also offer important insights into the biochemical mechanisms underlying this protective effect. Consequently, our study highlights potential new pharmacological targets aimed at preserving RBC function and tissue perfusion under conditions of increased oxidative stress.

The biconcave disc morphology of erythrocytes optimizes oxygen and carbon dioxide exchange by maximizing surface area. The high flexibility and deformability of the cell membrane enable erythrocytes to withstand mechanical stress as they pass through narrow vessels such as capillaries, thereby enhancing microcirculation efficiency.[18]

Numerous studies have demonstrated the detrimental effects of ischemia and subsequent reperfusion injury in skeletal muscle on RBC deformability, primarily due to oxidative stress and impaired redox balance in blood tissue.[17,19]

Similarly, in our study, ischemia-reperfusion injury was associated with a significant increase in the erythrocyte deformability index. An increased deformability index indicates impaired bending and twisting capacity of erythrocytes. In addition to the deformability index evaluation, structural alterations in erythrocytes were clearly observed using May-Grünwald and Giemsa staining. In rats treated with rosmarinic acid, the lower deformability index and significant reduction in structural abnormalities on MGG staining strongly suggest a protective effect of rosmarinic acid on erythrocytes.

Additionally, in our study, plasma MDA levels were evaluated to further assess erythrocyte damage. Unsaturated fatty acids, which are abundant in erythrocyte membranes, are the

most susceptible macromolecules to oxidative damage.[20]

Lipid peroxidation is a chain reaction initiated by free radicals and sustained by the abstraction of hydrogen atoms from polyunsaturated fatty acids. Lipid peroxides, such as MDA, serve as both markers and intermediates of lipid peroxidation and are commonly used to assess tissue damage caused by free radicals generated during ischemia-reperfusion.[21] In our study, plasma MDA levels were significantly higher in the IR group compared to the other groups, indicating that oxidative damage in erythrocytes increased as a result of IR exposure. Interestingly, in the group treated with both ischemia-reperfusion and rosmarinic acid (IR+RA), MDA levels were similar to those observed in the RA-C, DMSO, and Control groups. This finding supports the hypothesis that rosmarinic acid has a protective effect against oxidative damage and the resulting impairment of erythrocyte deformability.

It is well established that endothelial nitric oxide synthase, found in endothelial cells, plays a critical role in regulating vascular tone and, consequently, blood pressure. Interestingly, erythrocytes also contain a catalytically active isoform of eNOS. However, the physiological role of eNOS in RBCs remains unclear and is still under investigation.[16] Furthermore, various studies have demonstrated that nitric oxide can influence erythrocyte deformability.[22,23] During ischemia-reperfusion injury, peroxynitrite ($ONOO^-$), a highly reactive species, is formed by the reaction between nitric oxide and superoxide anion (O_2^-). Peroxynitrite contributes significantly to the pathophysiology of reperfusion injury by causing tissue damage through mechanisms such as lipid peroxidation, protein nitration, and DNA damage.[24]

However, there are studies in the literature suggesting that, under specific physiological conditions, nitric oxide exerts a protective effect on erythrocyte deformability.[23] Available data indicate that while nitric oxide is reduced by reactive oxygen radicals under oxidative stress, it also contributes to oxidative stress. Nevertheless, when applied independently, NO demonstrates a protective effect on erythrocytes. In our study, endothelial NO synthase expression was found to be increased in erythrocytes in the IR group, suggesting that NO production in erythrocytes rises during IR injury, exacerbating oxidative stress. Interestingly, despite high eNOS expression in the IR+RA group, a decrease in the erythrocyte deformability index was observed. This seemingly contradictory result may be explained by the relationship between NO's potential protective effect on erythrocytes and superoxide dismutase activity. SOD is an enzyme that catalyzes the dismutation of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2). It serves as the first line of defense against reactive oxygen species (ROS)-mediated damage and plays a critical role in mitigating diseases associated with oxidative stress.[25] In our study, SOD levels were found to be higher in the IR+RA group compared to other groups. Based on these findings, it is suggested that rosmarinic acid increases plasma SOD levels, thereby reducing the interaction between NO and reactive oxygen radicals, allowing the protective effects of NO on RBCs to become more pronounced.

CONCLUSION

The results of our study demonstrate that rosmarinic acid protects erythrocytes from deformability impairment caused by oxidative stress during ischemia-reperfusion injury. Its protective effects on erythrocyte deformability appear to be mediated by increasing superoxide dismutase activity, thereby preventing the conversion of nitric oxide into reactive anions and allowing nitric oxide to exert its beneficial effects. These findings suggest that rosmarinic acid could be a promising therapeutic agent for preserving erythrocyte function. However, further studies are necessary to determine the optimal dose and timing of rosmarinic acid administration. Additionally, more research is needed to evaluate its long-term safety and potential side effects before clinical application.

Limitations

Statistical analysis indicated that a larger sample size of experimental animals would enhance the robustness of the statistical evaluation. Future studies should aim to increase the number of experimental animals to address this limitation.

Ethics Committee Approval: Ethical approval was obtained from the Kobay DHL A.S. LocaL Ethical committee in Ankara, Türkiye (Date: 08.12.2023, Decision No: 694).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: C.G., H.K., E.D., Ş.K.; Design: C.G., E.D., B.S.Ö., Ş.K.; Supervision: C.G., E.D., B.S.Ö., Ş.K.; Resource: H.K., B.S.Ö., F.M.Ç., T.D.; Materials: F.M.Ç., G.E., G.A., T.Ö.; Data Collection and/or Processing: F.M.Ç., G.E., G.A., T.Ö.; Analysis and/or Interpretation: G.E., G.A., Y.T., V.C.Ö.; Literature Review: T.Ö., M.E.Ö., I.T.Ö., V.C.Ö.; Writing: T.Ö., M.E.Ö., I.T.Ö., Y.T.; Critical Review: T.Ö., M.E.Ö., I.T.Ö., Y.T., V.C.Ö.

Conflict of Interest: None declared.

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REFERENCES

1. Ozüyaman B, Grau M, Kelm M, Merx MW, Kleinbongard P. RBC NOS: Regulatory mechanisms and therapeutic aspects. Trends Mol Med 2008;14:314–22. [CrossRef]
2. Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, et al. An estimation of the number of cells in the human body. Ann Hum Biol 2013;40:463–71. Erratum in: Ann Hum Biol 2013;40:471. [CrossRef]
3. Barbarino F, Wäschenbach L, Cavalho-Lemos V, Dillenberger M, Becker K, Gohlke H, et al. Targeting spectrin redox switches to regulate the mechanoproperties of red blood cells. Biol Chem 2020;402:317–31. [CrossRef]
4. Kobayashi J, Ohtake K, Murata I, Sonoda K. Nitric oxide bioavailability for red blood cell deformability in the microcirculation: A review of recent progress. Nitric Oxide 2022;129:25–9. [CrossRef]
5. Kartal H, Comu FM, Kucuk A, Polat Y, Dursun AD, Arslan M. Effect of apelin-13 on erythrocyte deformability during ischaemia-reperfusion

injury of heart in diabetic rats. Bratisl Lek Listy 2017;118:133–6. [CrossRef]
6. Huseyin S, Reyhancan A, Halici U, Guclu O, Tuysuz S, Oztorun B, et al. Comparison of the protective effects of vanillic and rosmarinic acid on cardiac tissue: Lower limb ischemia-reperfusion model in rats. Ulus Travma Acil Cerrahi Derg 2024;30:619–25. [CrossRef]
7. Petersen M, Abdullah Y, Benner J, Eberle D, Gehlen K, Hücherig S, et al. Evolution of rosmarinic acid biosynthesis. Phytochemistry 2009;70:1663–79. [CrossRef]
8. Petersen M, Simmonds MS. Rosmarinic acid. Phytochemistry 2003;62:121–5. [CrossRef]
9. Hitl M, Kladar N, Gavarić N, Božin B. Rosmarinic acid-human pharmacokinetics and health benefits. Planta Med 2021;87:273–82. [CrossRef]
10. U.S. National Library of Medicine. Rosmarinic acid. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/5281792. Accessed March 3, 2025. [CrossRef]
11. Ramalho LN, Pasta ÂA, Terra VA, Augusto M, Sanches SC, Souza-Neto FP, et al. Rosmarinic acid attenuates hepatic ischemia and reperfusion injury in rats. Food Chem Toxicol 2014;74:270–8. [CrossRef]
12. Kartal H, Comu FM. Effect of melatonin on erythrocyte deformability in mice with ischemia-reperfusion injury in skeletal muscle. J Surg Res 2020;3:262–71. [CrossRef]
13. Arslan M, Comu FM, Isik B, Unal Y, Cekmen N, Kurtipek O. Effects of the general anaesthetic agent, propofol, on erythrocyte deformability. Bratisl Lek Listy 2010;111:126–8. [CrossRef]
14. Široká M, Franco C, Gulašová 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]
15. 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]
16. 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. [CrossRef]
17. 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]
18. Hall JE. Red blood cells, anemia, and polycythemia. In: Guyton and Hall textbook of medical physiology. 13th ed. Philadelphia: Elsevier Health Sciences; 2015. p. 445–54. [CrossRef]
19. Çomu FM, Kılıç Y, Özer A, Kirişçi M, Dursun AD, Tatar T, et al. Effect of picroside II on erythrocyte deformability and lipid peroxidation in rats subjected to hind limb ischemia reperfusion injury. Drug Des Devel Ther 2016;10:927–31. [CrossRef]
20. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 1991;288:481–7. [CrossRef]
21. Grisham MB, Granger DN. Free radicals: reactive metabolites of oxygen as mediators of postischemic reperfusion injury. In: Martson A, Bulkley GB, Fiddian-Green RG, Haglung U, editors. Splanchnic ischemia and multiple organ failure. St. Louis: Mosby; 1989. p.135–144. [CrossRef]
22. Belanger AM, Keggi C, Kanias T, Gladwin MT, Kim-Shapiro DB. Effects of nitric oxide and its congeners on sickle red blood cell deformability. Transfusion 2015;55:2464–72. [CrossRef]
23. Bor-Kucukatay M, Wenby RB, Meiselman HJ, Baskurt OK. Effects of nitric oxide on red blood cell deformability. Am J Physiol Heart Circ Physiol 2003;284:H1577–84. [CrossRef]
24. Özer MK, Çiçek E, Gökalp O, Koyu A, Parlakpınar H, Acet A. Role of nitric oxide in the renal damage after myocardial ischemia reperfusion and effect of caffeic acid phenethyl ester (cape). [Article in Turkish] SDÜ Tıp Fak Derg 2005;12:23–7.
25. Zhao H, Zhang R, Yan X, Fan K. Superoxide dismutase nanozymes: An emerging star for anti-oxidation. J Mater Chem B 2021;9:6939–57. [CrossRef]

DENEYSEL ÇALIŞMA - *ÖZ*

Sıçan alt ekstremite iskelet kası iskemi reperfüzyon hasarında rosmarinik asitin kırmızı kan hücresi deformabilitesi, morfolojisi ve nitrik oksit üzerindeki etkilerinin değerlendirilmesi

AMAÇ: Eritrosit deformabilitesi eritrositlerin kapiller damarlardan geçerken yaptıkları eğilip bükülme hareketidir ve doku perfüzyonu için hayati öneme sahiptir. Çalışmamızda alt ekstremite iskemi-reperfüzyon uygulanan sıçanlarda rosmarinik asit tedavisinin eritrosit deformabilitesini nasıl ve hangi yolak üzerinden etkilediği araştırılmıştır.
GEREÇ VE YÖNTEM: Çalışmamızda ağırlıkları 400-450 gr arasında değişen wistar albino sıçanlar kullanıldı. Sıçanlar rastgele 5 gruba ayırıldı. Herhangi bir işlem uygulanmayan kontrol grubu (C), yalnızca çözücü madde dimetil sülfoksit (DMSO) verilen grup (DMSO), alt ekstremie ana femoral arterden 90 dakika iskemi ve 90 dakika reperfüzyon uygulanan grup (IR), rosmarinik asitin tek başına etkilerinin incelendiği rosmarinik asit kontrol grubu (RA-C) ve iskemi reperfüzyon prosedüründen 1 saat önce intraperitoneal rosmarinik asit verilen grup (IR+RA). Prosedür bitiminde sıçanlardan intrakardiak kan alınarak May-Grunwald ve Giemsa (MGG), endotelial nitrik oksit sentaz (eNOS), eritrosit deformabilite indeksleri, malondialdehit (MDA) ve süperoksit dismutaz (SOD) düzeyleri çalışıldı.
BULGULAR: Çalışma sonuçlarında anlamlı değişiklikler saptandı. İskemi reperfüzyon öncesi rosmarinik asit verilen grupta eritrosit deformabilitesi sadece iskemi reperfüzyon yapılan gruba göre istatiksel anlamlı olarak daha iyi tespit edildi. Eritrositlerin morfolojik değişiklikleri de IR+RA grubunda IR grubuna göre istatiksel anlamlı olarak daha iyi bulundu. İmmünohistokimyasal eNOS boyamasında ise IR grubundaki eNOS aktivitesinin IR+RA grubuna göre daha yüksek düzeyde olduğu tespit edildi. Malondialdehit (MDA) ölçümlerinde IR grubunun MDA ölçümlerinin diğer gruplara göre anlamlı düzeyde yüksek olduğu izlendi. Süperoksit dismutaz (SOD) düzeyi analizinde IR-RA grubunun SOD ölçümlerinin diğer gruplara göre anlamlı düzeyde yüksek olduğu izlendi.
SONUÇ: Çalışma sonuçlarımızda iskemi öncesi uygulanan rosmarinik asit tedavisinin eritrosit deformasyonunu ve eritrositlerin morfolojik bozulmalarına karşı koruyucu özelliğinin olduğunu gösterdi. Deformabiliteyi düzeltmedeki yolunun ise SOD düzeyini arttırarak, nitrik oksit (NO) üretiminin yan ürünleri olan reaktif oksijen anyonlarını indirgemesi, antioksidan etki oluşturması ve bu şekilde NO'in faydalı etkilerinden yararlanılabilmesi yoluyla olabileceği öngörüldü.

Anahtar sözcükler: Rosmarinik asit; iskemi reperfüzyon; eritrosit deformabilite; nitrik oksit.

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