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Original Article

The effect of silymarin on ischemia-reperfusion injury in skeletal muscles of rats

Abdullah Ozer[1], Basak Kocak[1], Mustafa Arslan[2], Baris Mardin[1], Aysegul Kucuk[3], Saban Cem Sezen[4], Mustafa Hakan Zor[1], Mustafa Kavutcu[5]

[1]Gazi University, Faculty of Medicine, Department of Cardiovascular Surgery, Ankara, Türkiye
[2]Gazi University, Faculty of Medicine, Department of Anaesthesiology and Reanimation, Ankara, Türkiye
[3]Kütahya Health Sciences University, Faculty of Medicine, Department of Physiology, Kütahya, Türkiye
[4]Kırıkkale University, Faculty of Medicine, Department of Histology and Embryology, Kırıkkale, Türkiye
[5]Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Türkiye

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Abstract

Aim: Although the precise process is not entirely elucidated, it is well-known that oxidative stress mediators contribute to ischemia-reperfusion (I/R) injury. The impact of silymarin on I/R injury in many different tissues has been examined. For this purpose, we planned to see the effect of silymarin on muscle tissue in rats subjected to lower extremity I/R injury.

Material and Methods: We used 18 male Wistar albino rats weighing 225-275 g. Each of the three groups of rats [Control (C), Ischemia-Reperfusion (I/R), and Silymarin-Ischemia/Reperfusion (S-I/R)] consisted of six rats. Silymarin was administered intraperitoneally 30 minutes before the procedure. (100 mg/kg-1) In Group I/R, the infrarenal abdominal aorta was clamped with a microvascular clamp. The clamp was removed after 120 minutes, and reperfusion was achieved for the next 120 minutes. At the end of the reperfusion period, muscle tissue samples were collected, and Malondialdehyde (MDA), catalase (CAT) enzyme activity levels and histopathological parameters were compared.

Results: In the histopathological examination, no degeneration was observed in the muscle fibers in the Group C, while findings of striated muscle damage such as muscle atrophy/hypertrophy, muscle degeneration/congestion, internalization of the muscle nucleus/oval/central nucleus, fragmentation/hyalinization and leukocyte cell infiltration were seen in the Group I/R. In Group S-I/R, muscle atrophy /hypertrophy, internalization of the muscle nucleus/oval/central nucleus, fragmentation/hyalinization, and leukocyte cell infiltration were observed to improve these damaged areas compared to Group I/R. MDA levels in the Group I/R were significantly higher compared to Group C and S-I/R. The activity of the CAT enzyme was much higher in Group I/R compared to Group C.

Conclusion: Our study revealed that 100 mg/kg-1 silymarin administered by intraperitoneal injection 30 minutes before ischemia effectively decreased lipid peroxidation, oxidative stress, and the injury caused by I/R in muscle histology in rats.

Keywords: Silymarin, malondialdehyde, catalase, ischemia-reperfusion

INTRODUCTION

Ischemia is an insufficient oxygen supply to tissues or organs due to inadequate blood flow. It can occur when blood flow is reduced. Ischemia leads to cell death as it reduces the stored cellular energy and causes the accumulation of harmful substances [1]. Ischemia-reperfusion (I/R) injury implies the return of oxygenated blood to an area of tissue previously affected by ischemia [2]. It makes cell structure damaged

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Corresponding Author: Mustafa Arslan, Gazi University, Faculty of Medicine, Department of Anaesthesiology and Reanimation, Ankara, Türkiye
Email: mustarslan@gmail.com

due to cell apoptosis and necrosis [3,4]. I/R injury causes the reactive oxygen species (ROS) to be released. ROS mainly attacks the polyunsaturated fatty acids located in the cell membrane. Lipid peroxide radicals are generated due to the interaction between fatty acids and ROS. Metals found in the environment can act as catalysts in reactions involving lipid peroxides, leading to the production of breakdown products such as propanal, hexanal, 4-hydroxynonenal, and malondialdehyde (MDA) [5].

Previous studies indicate that the catalase (CAT) enzyme, produced by various genes in plants, belongs to the enzymatic antioxidant class. It shows an essential antioxidant effect due to its ability to efficiently neutralize ROS and its involvement in the catalysis of hydrogen peroxide [6,7]. Many studies have investigated I/R injury to find an effective agent to protect cells and their components. One of these agents is silymarin, a compound molecule isolated from Silybum marianum [8]. Its molecular structure combines flavonolignans, flavonoids, fatty acids, and polyphenolic compounds [9]. Older studies have established silymarin's hepatoprotective effect [10] and new research examining its impact on other structures is underway. Silybin is widely acknowledged as the primary and most potent ingredient of silymarin.

Despite various research about silymarin and its effect on different tissues, to our knowledge, its relationship with lower limb skeletal muscle under I/R injury is quite rare. Our study aimed to show the protective effect of silymarin on lower limb skeletal muscle I/R injury.

MATERIAL AND METHODS

Study Protocol and Animals

This study was approved by our center's ethics committee (Ethic number: G.U.ET-19-044). The standards of the Guide for the Care and Use of Laboratory Animals were accepted for procedures. At the onset of experimental procedures, all rats were anesthetized with ketamine (50 mg/kg, intraperitoneal) and xylazine (10 mg/kg, intraperitoneal). During the surgical procedure, rats were placed on a heating pad to maintain the body temperature. Rats were kept in a temperature-controlled (21±1°C) and humidity-controlled room (45–55%) maintained on a 12/12 reversed light cycle. They were fed a standard pellet and allowed to drink water ad libitum.

After examining the literature, a total of 18 Wistar albino rats weighing between 270 and 300 grams were used for the current study. Animals were equally divided into three equal groups (n=6): Group C, Group I/R, and Group S-I/R. Midline laparotomy was the surgical procedure in Group C and there was no treatment. In Group I/R, I/R injury without treatment, a midline laparotomy was performed, and the infrarenal aorta was left clamped for 2 hours. Reperfusion was established for the following 2 hours by removing the clamp. After the identical procedure as Group I/R, silymarin was given (100 mg.kg-1) intraperitoneally 30 minutes before the ischemia period in Group S-I/R (I/R injury with silymarin treatment). Gastrocnemius muscle tissue samples were collected, and subjects were sacrificed by taking intraabdominal blood and applying a lethal dose of ketamine at the end of 4 hours.

The histopathological examination investigated muscle atrophy/hypertrophy, degeneration/congestion, internalization of muscle nuclei with oval/central nucleus, fragmentation/hyalinization, and leukocyte cell infiltration. We assessed hypertrophic alterations using a 4-point scoring system. The scoring system ranged from 0 (-), indicating no changes, 1+ for minimal changes, 2++ for moderate changes, and 3+++ for significant changes. As a result of the evaluation, the following data were obtained for statistical analysis. Additionally, MDA levels and CAT enzyme activity were detected for biochemical and statistical assessment.

Statistical Analysis

The data were analyzed with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 22.0 program for Windows statistical software. The Kruskal-Wallis test was applied to assess biochemical and histological parameters. A p-value less than 0.05 was considered to be statistically significant.

RESULTS

Histopathological Results

The muscle fiber is a multi-nucleated, syncytial-like structure that resembles a long, narrow tube with the sarcoplasm showing a subtle, light pink coloring. The sarcolemmal nuclei are elongated and thin, located in parallel with the longitudinal axis of the fiber. Group S-I/R presents a similarly light microscopic morphology to Group C, attributed to the protective properties of silymarin. Remarkable differences between the groups were seen in terms of muscle atrophy/hypertrophy (p=0.015), muscle degeneration/congestion (p=0.085), internalization of muscle nuclei -oval/central nucleus (p=0.012), fragmentation /hyalinization (p=0.070), and leukocyte cell infiltration (p=0.075). The Group I/R exhibited more occurrence of muscle atrophy/hypertrophy than the Group C (p=0.002) (Table 1, Figure 1). Moreover, muscle atrophy/hypertrophy was markedly reduced in the Group S-I/R compared to the Group I/R (p=0.015). The Group I/R demonstrated a higher internalization of muscle nuclei -oval/central nucleus than the Group C (p=0.001). The Group S-I/R exhibited lower internalization of muscle nuclei -oval/central nucleus than the Group I/R (p=0.047).

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Table 1. Muscle tissue histopathological data (Mean±SE)

	Group C (n=6)	Group I/R (n=6)	Group S-I/R (n=6)	P**
Muscle atrophy-hypertrophy	0.33±0.21*	1.50±0.22	0.67±0.21*	0.015
Muscle degeneration- congestion	0.33±0.21	1.33±0.42	0.67±0.21	0.085
Internalisation of muscle nuclei -oval/central nucleus	0.17±0.17*	1.50±0.22	0.83±0.31*	0.012
Fragmentation -hyalinization	0.33±0.21	1.50±0.42	0.83±0.31	0.070
Leukocyte cell infiltration	0.50±0.22	1.17±0.31	0.33±0.21	0.075

C: control, I/R: ischemia/reperfusion, S-I/R: silymarin-ischemia/reperfusion; P **significance level with Kruskal Wallis test p<0.05; *p<0.05: compared to group I/R

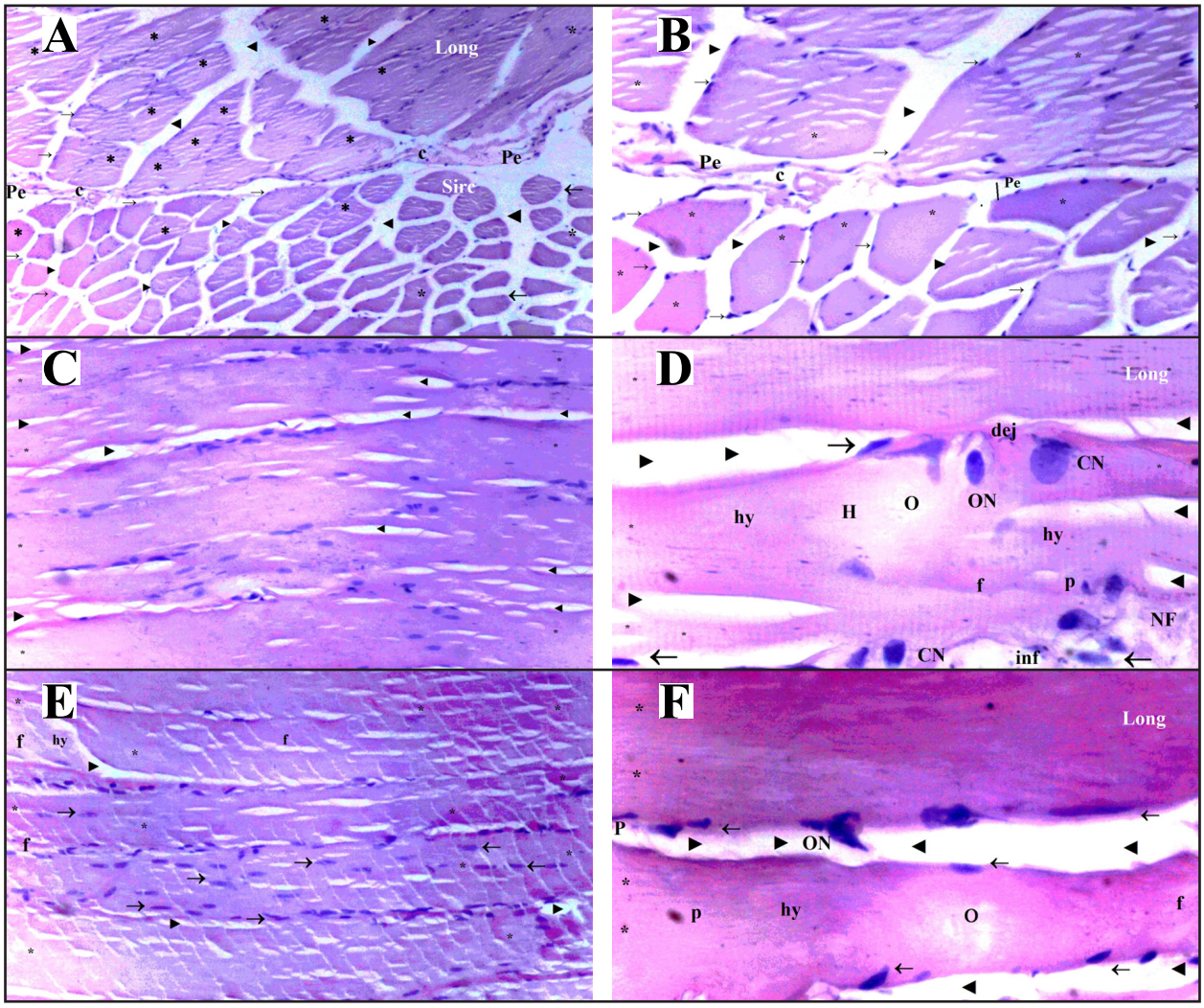

Figure 1. The muscle fiber is a multi-nucleated, syncytial-like structure that resembles a long, narrow tube; the sarcoplasm shows a subtle, light pink coloring; the sarcolemmal nuclei are elongated and thin, located in parallel with the longitudinal axis of the fiber; group S-I/R presents a similarly light microscopic morphology to Group C, attributed to the protective properties of silymarin; **1A.** skeletal muscle longitudinal section light microscopy for the Control group (H&E: hematoxylin and eosin X40); **1B.** skeletal muscle longitudinal section light microscopy for the Control group (H&E: hematoxylin and eosin X100); **1C.** skeletal muscle longitudinal section, group I/R (H&E: hematoxylin and eosin X40); **1D.** skeletal muscle longitudinal section light microscopy for the I/R group (H&E: hematoxylin and eosin X400); **1E.** skeletal muscle longitudinal section light microscopy for the S-I/R group (H&E: hematoxylin and eosin X40)); **1F.** skeletal muscle longitudinal section light microscopy for the S-I/R group (H&E: hematoxylin and eosin X400); →: peripheric nucleus, *: muscle fibers, ►: intercellular space, Dej: degeneration, CN: central nucleus, ON: oval nucleus, H: hypertrophy, Hy: hyalinization, F: fragmentation, inf: inflamation, NF: necrotic fibrils, P: picnotic nucleus h: hyalinization

Biochemical Results

The MDA levels significantly differed between the groups (p=0.037). The MDA levels were higher in the Group I/R than in the Group C (p=0.041). Moreover, significantly reduced MDA levels were found in the Group S-I/R compared to the Group I/R (p=0.017) (Table 2). CAT enzyme activity in the gastrocnemius muscle tissues significantly differed between the groups (p=0.049). The Group I/R showed higher CAT enzyme activity than the Group C (p=0.018).

Table 2. Muscle tissue MDA and CAT enzyme activity data (mean±SE)

	Group C (n=6)	Group I/R (n=6)	Group S-I/R (n=6)	P**
CAT (IU/mg.protein)	499.00±49.81	687.67±60.95*	564.00±36.37	0.049
MDA (nmol/mg.protein)	1.18±0.11*	1.72±0.12	1.07±0.24*	0.037

MDA: malondialdehyde, CAT: catalase; P **significance level with Kruskal Wallis test p<0.05; *p<0.05: compared to group I/R

DISCUSSION

We hypothesized that silymarin has a protective effect on I/R injury of lower limb muscle. In some clinical circumstances, such as transplant surgery, it is widely accepted that immunosuppressive treatments are commonly used to suppress the immune response. However, using immunosuppressive medication is not suitable in cardiac or vascular surgery, not regularly at least. These major discrepancies might help to understand the reason for much research about various pharmacological agents that can be used in I/R injury. In this regard, many newly discovered agents will be helpful for literature, especially for lower limb I/R injury. Our results supported this theory. The anti-inflammatory activity of silymarin is commonly accepted [11,12]. Furthermore, silymarin exerts its cell-protective effects through its antioxidative and radical scavenging activities and by interacting with particular receptors such as estrogen receptors, nuclear receptors, and P glycoproteins [13]. The CAT enzyme catalyzes the reduction of hydrogen peroxide molecules in different tissues' peroxisomes. Inadequate tissue levels of catalase following I/R injury might impede protection against the harmful effects of hydrogen peroxide resulting in cellular damage. Prior research has demonstrated that silymarin has a preventive impact on I/R injury by augmenting the activity of CAT [14]. In our study, we measured MDA levels as a lipid peroxidation marker after IR injury and found significantly decreasing MDA levels; we believe that is a possible mechanism of silymarin as an inhibitor of lipid peroxidation. With its historical importance in an enormous field from Europe to Asia, Silybum marianum and its derivated form, silymarin, are the main topics because of their therapeutic efficacy in different clinical studies [11,15-19]. (Sigma-Aldrich; Merck KGaA; cat. no. SO292-50G). In our study, we used silymarin with more than 30% silybin content. However, though many studies have shown silymarin to be clinically reliable and well-tolerated, there are also contrasting results [17,20,21]. The research published by Schrieber et al. [20] found gastrointestinal and neurological side effects. However, other investigations in the literature have shown no adverse events, even when using equal or greater dosages [21,22]. Several studies have emphasized that this anti-inflammatory activity of silymarin may be dose-dependent, especially while it inhibits interferon g, IL 4, and IL 10 [13,23-25]. In light of these, it is possible to accept that the effectiveness of silymarin preparations may be influenced by the chemical variations in their content [10]. The main limitation of our study was the number of subjects. Another point that should be considered is silymarin dosage.

CONCLUSION

In conclusion, our research showed that silymarin has a protective effect on lower limb extremity muscle I/R injury. It augments the activity of CAT and decreases MDA by inhibiting lipid peroxidation. Based on previous studies, the effectiveness of silymarin may depend on dosage and types of compound. Hence, the authors of this manuscript believe that it is important to investigate the effects of silymarin on various organs and tissues in future research prior to its application in medical conditions.

Ethics Committee Approval: Ethical approval for the study was obtained from Animal Research Committee of Gazi University (Ankara, Turkey; approval no. G.Ü.ET-19.044).

Patient Consent for Publication: Patient consent form is not required.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Köksal Z, Kurtipek Ö, Arslan M, Dursun AD, Yığman Z, Özer A. Protective effects of hydrogen rich saline solution in rats with experimental myocardial ischemia reperfusion injury. Heliyon. 2023;9:e22973.

2. Özer A, Koçak B, Sezen ŞC, Arslan M, Kavutçu M. The Effect of "Proanthocyanidin" on Ischemia-Reperfusion Injury in Skeletal Muscles of Rats. Medicina (Kaunas). 2024;60:804.

3. Yan HF, Tuo QZ, Yin QZ, Lei P. The pathological role of ferroptosis in ischemia/reperfusion-related injury. Zool Res. 2020;41:220-30.

4. Draganovic D, Lucic N, Jojic D. Oxidative stress marker and pregnancy induced hypertension. Med Arch. 2016;70:437-40.

5. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438.

6. He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cell Physiol Biochem. 2017;44:532-53.

7. Zandi P, Schnug E. Reactive oxygen species, antioxidant responses and implications from a microbial modulation perspective. Biology (Basel). 2022;11:155.

8. Rao PR, Viswanath RK. Cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial infarction in albino rats. Exp Clin Cardiol. 2007;12:179-87.

9. Surai PF. Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. Antioxidants (Basel). 2015;4:204-47.

10. Comelli MC, Mengs U, Schneider C, Prosdocimi M. Toward the definition of the mechanism of action of silymarin: Activities related to cellular protection from toxic damage induced by chemotherapy. Integr Cancer Ther. 2007;6:120-9.

11. Camini FC, Costa DC. Silymarin: not just another antioxidant. J Basic Clin Physiol Pharmacol. 2020;31:/j/jbcpp.2020.31.issue-4/jbcpp-2019-0206/jbcpp-2019-0206.xml.

12. Aghazadeh S, Amini R, Yazdanparast R, Ghaffari SH. Anti-apoptotic and anti-inflammatory effects of Silybum marianum in treatment of experimental steatohepatitis. Exp Toxicol Pathol. 2011;63:569-74.

13. Saller R, Melzer J, Reichling J, Brignoli R, Meier R. An updated systematic review of the pharmacology of silymarin. Forsch Komplementmed. 2007;14:70-80.

14. Elsayed AI, El-Hamahmy MAM, Rafudeen MS, Mohamed AH, Omar AA. The impact of drought stress on antioxidant responses and accumulation of flavonolignans in milk thistle *(Silybum marianum (l.) gaertn)*. Plants (Basel). 2019;8:611.

15. Hadi A, Pourmasoumi M, Mohammadi H, Symonds M, Miraghajani M. The effects of silymarin supplementation on metabolic status and oxidative stress in patients with type 2 diabetes mellitus: A systematic review and meta-analysis of clinical trials. Complement Ther Med. 2018;41:311-9.

16. Koltai T, Fliegel L. Role of silymarin in cancer treatment: facts, hypotheses, and questions. J Evid Based Integr Med. 2022;27:2515690X211068826.

17. Soleimani V, Delghandi PS, Moallem SA, Karimi G. Safety and toxicity of silymarin, the major constituent of milk thistle extract: an updated review. Phytother Res. 2019;33:1627-38.

18. Abenavoli L, Izzo AA, Milić N, Cicala C, Santini A, Capasso R. Milk thistle (Silybum marianum): a concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. Phytother Res. 2018;32:2202-13.

19. Tighe SP, Akhtar D, Iqbal U, Ahmed A. Chronic liver disease and silymarin: a biochemical and clinical review. J Clin Transl Hepatol. 2020;8:454-8.

20. Schrieber SJ, Hawke RL, Wen Z, Smith PC, Reddy KR, Wahed AS, et al. Differences in the disposition of silymarin between patients with nonalcoholic fatty liver disease and chronic hepatitis C. Drug Metab Dispos. 2011;39:2182-90.

21. Fried MW, Navarro VJ, Afdhal N, Belle SH, Wahed AS, Hawke RL, et al. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: a randomized controlled trial. JAMA. 2012;308:274-82.

22. Shahbazi F, Sadighi S, Dashti-Khavidaki S, Shahi F, Mirzania M, Abdollahi A, et al. Effect of silymarin administration on cisplatin nephrotoxicity: report from a pilot, randomized, double-blinded, placebo-controlled clinical trial. Phytother Res. 2015;29:1046-53.

23. Surai PF, Surai A. Silymarin and inflammation: from understanding molecular mechanisms to practical applications. In: Silymarin Puzzle Surai PF, Surai A, eds. Wageningen Academic Publishers: Wageningen, The Netherlands; 2023, p. 287-317.

24. Sharma S, Kumar P, Ashawat MS, Pandit V, Verma CS, Sharma DK. Silymarin: a phytoconstituent with significant therapeutic potential a narrative review. Curr Drug Ther. 2023;18:89-97.

25. Yavuz A, Küçük A, Ergörün Aİ, Dursun AD, Yiğman Z, Alkan M, Arslan M. Evaluation of the efficacy of silymarin and dexmedetomidine on kidney and lung tissue in the treatment of sepsis in rats with cecal perforation. Exp Ther Med. 2024;27:242.