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Time-dependent protective effects of Silymarin on renal Ischemia-reperfusion injury in rats

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Abstract

Objective: Renal ischemia–reperfusion (I/R) injury is a major cause of acute kidney injury observed in surgical and clinical settings such as shock, trauma, partial nephrectomy, and renal transplantation. This study aimed to evaluate the protective effects of silymarin, a flavonolignan complex extracted from Silybum marianum (milk thistle), against renal I/R injury at varying ischemia durations in rats.

Materials and methods: Forty-eight adult male Wistar albino rats were randomly divided into eight groups (n=6 each). The sham group underwent laparotomy only. Control I/R groups were subjected to 45, 60, or 90 minutes of ischemia followed by 180 minutes of reperfusion. Silymarin-treated groups received 100 mg/kg/day silymarin orally for seven days before surgery and underwent the same I/R protocols. At the end of reperfusion, renal tissues and blood samples were collected to determine total antioxidant status (TAS), total oxidant status (TOS), superoxide dismutase (SOD), and malondialdehyde (MDA) levels. Histopathological evaluation was performed on hematoxylin–eosin–stained sections.

Results: The highest serum TAS and SOD levels were observed in the SIL 45 group, indicating a strong early antioxidant response to silymarin. Serum and tissue MDA levels, indicators of lipid peroxidation, were significantly lower in silymarin-treated groups than in corresponding I/R groups. However, the protective effect decreased as ischemia duration increased. Histopathological analysis revealed less tubular necrosis, vacuolar degeneration, and interstitial congestion in silymarin-treated groups, particularly at shorter ischemia durations.

Conclusion: Silymarin exerts a significant renoprotective effect by enhancing antioxidant capacity and reducing oxidative stress in renal I/R injury. Nevertheless, its efficacy diminishes with prolonged ischemia, suggesting a time-dependent limitation in protection.

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Introduction

Renal ischemia–reperfusion (I/R) injury is a critical pathophysiological event that contributes to acute kidney injury (AKI) in both surgical and clinical settings. It occurs frequently in conditions such as shock and sepsis, as well as during partial nephrectomy, kidney transplantation, and vascular surgeries. Temporary occlusion of the renal pedicle is often required during surgical procedures to prevent intraoperative bleeding and ensure a clear operative field. However, prolonged ischemia followed by reperfusion results in the generation of reactive oxygen species (ROS), inflammation, and cellular apoptosis, which collectively lead to structural and functional renal impairment (1).

During the ischemic phase, hypoxia interrupts oxidative phosphorylation, resulting in ATP depletion and disruption of ionic homeostasis. Reperfusion paradoxically exacerbates cellular injury by generating ROS through the activation of xanthine oxidase and inflammatory leukocytes. The resulting oxidative stress triggers lipid peroxidation, mitochondrial dysfunction, and activation of cell death pathways. This cascade is a key contributor to renal tubular necrosis and loss of renal function (1-3).

Several experimental strategies have been explored to prevent renal I/R injury, including ischemic preconditioning, pharmacologic antioxidant therapy, and the use of free radical scavengers. In recent years, natural antioxidant compounds have attracted increasing attention due to their safety and broad biological activities (4,5). Silymarin, a mixture of flavonolignans obtained from Silybum marianum (milk thistle), has demonstrated potent hepatoprotective, anti-inflammatory, and antioxidant properties (6). Its active components—silibinin, silychristin, and silydianin—act as free radical scavengers and upregulate antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (6,7).

Experimental studies have shown that silymarin attenuates oxidative damage in liver, myocardial, and pancreatic I/R injury models (8-10). However, its protective duration and efficacy under different renal ischemia times remain unclear. The present study aimed to evaluate the time-dependent renoprotective effects of silymarin on renal I/R injury in rats through biochemical and histopathological analyses. It was hypothesized that pretreatment with silymarin would enhance antioxidant capacity and reduce oxidative damage, although its protective efficacy might decline with prolonged ischemia.

Materials and methods

Animals and experimental design

This experimental study was approved by the Animal Ethics Committee of Kahramanmaras Sutcu Imam University (approval no.: 2014/01-11) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH). Forty-eight adult male Wistar albino rats (250–300 g) were housed under controlled laboratory conditions (24±2°C, 50±10% humidity, 12-hour light/dark cycle) with ad libitum access to food and water. The rats were randomly divided into eight groups (n=6 per group):

Anesthesia and surgical procedure

Rats were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg). After midline laparotomy, the left renal pedicle was carefully dissected and clamped with a nontraumatic vascular clamp to induce ischemia for the specified duration. Reperfusion was achieved by releasing the clamp and verifying restoration of blood flow. Sham-operated rats underwent identical procedures without clamping. At the end of reperfusion, blood samples were obtained via intracardiac puncture, and the left kidney was excised for biochemical and histopathological analysis.

Biochemical analyses

Renal tissues were homogenized in 1.15% KCl buffer and centrifuged at 10,000 × g for 30 min at 4°C. Supernatants were analyzed spectrophotometrically for total antioxidant status (TAS), total oxidant status (TOS), superoxide dismutase (SOD), and malondialdehyde (MDA) using commercial kits (RelAssay Diagnostics, Turkey). Results were expressed as mmol Trolox Eq/L for TAS, µmol H_2O_2 Eq/L for TOS, U/mg protein for SOD, and nmol/mg protein for MDA.

Statistical analysis

All statistical analyses were performed using SPSS version 17.0. Results were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using one-way ANOVA followed by Tukey's post hoc test. A $p < 0.05$ was considered statistically significant.

Table 1: Experimental design showing treatment regimen and ischemia–reperfusion (I/R) durations applied to each group.

Groups	Treatment / Intervention	Ischemia–Reperfusion Protocol
Sham	Laparotomy only; no ischemia or reperfusion	–
Saline + IR45	Isotonic saline (2 mL/day, orally) for 7 days	45 min ischemia + 180 min reperfusion
IR45	No treatment	45 min ischemia + 180 min reperfusion
SIL45	Silymarin (100 mg/kg/day, orally) for 7 days	45 min ischemia + 180 min reperfusion
IR60	No treatment	60 min ischemia + 180 min reperfusion
SIL60	Silymarin (100 mg/kg/day, orally) for 7 days	60 min ischemia + 180 min reperfusion
IR90	No treatment	90 min ischemia + 180 min reperfusion
SIL90	Silymarin (100 mg/kg/day, orally) for 7 days	90 min ischemia + 180 min reperfusion

IR: Ischemia–Reperfusion, SIL: Silymarin

Results

Biochemical findings

Serum and tissue oxidative stress parameters are summarized in Table 2. Serum TAS values were highest in the SIL45 group, indicating the most pronounced antioxidant response at shorter ischemia durations. In contrast, serum TAS levels declined progressively in the SIL60 and SIL90 groups, suggesting a time-dependent decrease in silymarin efficacy. Tissue TAS levels followed a similar pattern, with the highest mean value observed in the SIL45 group (1.49±0.35 nmol/mg protein).

SOD activity was significantly higher in the SIL45 group (2.31 U/mL) compared with corresponding IR controls, while longer ischemia durations resulted in a gradual reduction in SOD activity. Tissue SOD activity was also elevated in all silymarin-treated groups, with the highest value detected in SIL90 (26.9±42.9 nmol/mg protein).

Serum TOS was markedly elevated in the 90 IR group (9.50±5.88 µmol/L). In comparison, silymarin-treated groups exhibited lower serum TOS levels, particularly in SIL90, indicating partial suppression of oxidative stress.

Serum and tissue malondialdehyde (MDA) levels, a lipid peroxidation marker, were significantly reduced in all silymarin-treated groups compared with their respective IR counterparts ($p < 0.05$).

Histopathological findings

Histopathological evaluation revealed normal renal architecture in the sham group. In the IR groups, varying degrees of tubular necrosis, epithelial desquamation, and interstitial congestion were observed. Silymarin-treated groups showed a marked reduction in histopathological damage, characterized by decreased tubular cast formation, vacuolar degeneration, and medullary congestion. The most prominent protective effect was seen in the SIL45 group, where tubular morphology was largely preserved. Overall, both

Table 2: Serum oxidative stress parameters among experimental groups (compact view)

Group	TAS (mmol/L)	SOD (U/mL)	TOS (μmol/L)	MDA (nmol/mg protein)
Sham	0.58 ± 0.57	1.41 ± 0.20	6.80 ± 2.35	6.33 ± 1.27
Saline + IR45	0.77 ± 0.33	1.44 ± 0.31	4.42 ± 3.27	5.59 ± 0.70
IR45	1.19 ± 0.86	1.39 ± 0.34	6.12 ± 3.19	10.40 ± 4.97
SIL45	1.03 ± 1.00	1.60 ± 0.45	9.04 ± 6.28	6.03 ± 0.99
IR60	0.75 ± 0.53	1.28 ± 0.24	3.78 ± 1.65	18.35 ± 16.76
SIL60	0.23 ± 0.16	1.38 ± 0.38	7.29 ± 2.01	5.66 ± 0.91
IR90	1.00 ± 0.51	1.47 ± 0.24	9.51 ± 5.89	9.33 ± 5.45
SIL90	0.49 ± 0.21	1.15 ± 0.14	5.61 ± 2.48	5.45 ± 0.61

IR: Ischemia–Reperfusion, SIL: Silymarin, TAS: Total antioxidant status, TOS: Total oxidant status, SOD: superoxide dismutase, MDA: Malondialdehyde, Mean ± SD (standard deviation)

Table 3: Tissue oxidative stress parameters among experimental groups (compact view)

Group	TAS (μmol/mg protein)	SOD (μmol/mg protein)	TOS (μmol/mg protein)	MDA (μmol/mg protein)
Sham	1.39 ± 0.55	8.56 ± 2.59	13.24 ± 4.38	3.03 ± 1.39
Saline + IR45	0.67 ± 0.39	11.09 ± 3.69	17.81 ± 5.55	4.14 ± 1.44
IR45	1.00 ± 0.35	14.61 ± 6.92	11.16 ± 1.79	5.51 ± 1.79
SIL45	1.49 ± 0.35	7.26 ± 2.26	25.45 ± 10.34	5.58 ± 1.57
IR60	1.31 ± 0.31	13.67 ± 5.27	12.49 ± 2.38	5.29 ± 2.44
SIL60	1.17 ± 0.42	12.27 ± 8.13	15.94 ± 5.51	4.93 ± 2.28
IR90	1.28 ± 0.55	10.70 ± 2.20	11.77 ± 3.88	3.83 ± 1.35
SIL90	1.28 ± 0.32	26.92 ± 42.91	18.17 ± 5.38	4.65 ± 2.30

IR: Ischemia–Reperfusion, SIL: Silymarin, TAS: Total antioxidant status, TOS: Total oxidant status, SOD: superoxide dismutase, MDA: Malondialdehyde, Mean ± SD (standard deviation)

biochemical and histological analyses confirmed that silymarin exerted significant renoprotective effects against ischemia–reperfusion injury. However, the magnitude of protection decreased as the duration of ischemia increased, supporting the hypothesis of a time-dependent limitation in its efficacy.

Table 4: Histopathological findings and mean lesion scores in renal tissues

Group	Vacuolar degeneration	Tubular cast formation	Tubular dilatation	Medullary congestion
Sham	0.83 ± 0.41 (Mild)	0.83 ± 0.41 (Minimal)	0.67 ± 0.52 (Mild)	1.00 ± 0.00 (Mild)
Saline + IR45	1.50 ± 0.55 (Moderate)	1.83 ± 0.75 (Moderate)	1.50 ± 0.55 (Moderate)	2.17 ± 0.41 (Moderate)
IR45	1.50 ± 0.55 (Moderate)	1.00 ± 0.00 (Mild)	1.83 ± 0.75 (Moderate–Severe)	1.83 ± 0.75 (Moderate)
SIL45	1.33 ± 0.82 (Mild)	1.00 ± 0.89 (Mild)	1.33 ± 0.52 (Mild–Moderate)	2.50 ± 0.55 (Moderate–Severe)
IR60	1.50 ± 0.84 (Moderate)	2.50 ± 0.84 (Severe)	1.50 ± 0.55 (Moderate)	2.17 ± 0.75 (Moderate)
SIL60	1.33 ± 0.52 (Mild)	1.50 ± 0.84 (Moderate)	0.83 ± 0.41 (Mild)	1.67 ± 1.21 (Mild–Moderate)
IR90	1.67 ± 1.03 (Moderate–Severe)	2.00 ± 0.89 (Moderate–Severe)	1.83 ± 0.75 (Moderate–Severe)	2.17 ± 0.98 (Moderate–Severe)
SIL90	2.17 ± 0.41 (Moderate–Severe)	2.67 ± 0.52 (Severe)	1.67 ± 0.52 (Moderate)	2.50 ± 0.84 (Severe)

Histopathological lesion scores for vacuolar degeneration, tubular cast formation, tubular dilatation, and medullary congestion in renal tissues. Scores were graded semiquantitatively as follows: 0=none, 1=mild, 2=moderate, 3=severe.

Discussion

The present study demonstrated that silymarin exerts significant renoprotective effects against renal ischemia–reperfusion (I/R) injury in rats. The protective mechanism appears to be closely associated with the enhancement of antioxidant defense and suppression of oxidative stress. Biochemical analysis revealed that silymarin increased serum and tissue TAS and SOD activity while reducing TOS and MDA levels, indicating a restoration of redox balance. Histopathological evaluations were consistent with these biochemical findings, showing attenuated tubular necrosis, vacuolar degeneration, and interstitial congestion in the silymarin-treated groups, particularly at shorter ischemia durations. The pathophysiology of renal I/R injury involves the generation of ROS, lipid peroxidation, and the activation of inflammatory and apoptotic pathways. During reperfusion, the sudden influx of oxygen leads to excessive ROS formation, resulting in membrane damage, mitochondrial dysfunction, and loss of tubular integrity (1,3). Antioxidant systems such as SOD and catalase constitute the first line of defense against ROS-mediated cellular injury. In the present study, the elevated TAS and SOD levels in silymarin-treated groups suggest that the compound enhances endogenous antioxidant capacity, thereby counteracting oxidative injury. These findings are consistent with previous reports showing that silymarin improves renal function and attenuates oxidative damage in various experimental models of I/R injury and nephrotoxicity (8-12).

The reduction in MDA levels in silymarin-treated rats further confirms the inhibition of lipid peroxidation. Histopathological preservation of renal tissue architecture, especially in the SIL45 group, supports the biochemical evidence of antioxidant protection. However, the efficacy of silymarin was observed to decline with prolonged ischemia (60–90 minutes), suggesting a time-dependent limitation of its protective potential. This reduction may be attributed

to the overwhelming generation of ROS during extended ischemic periods, which surpasses the scavenging capacity of exogenous antioxidants (1,2,4).

Overall, these results indicate that silymarin confers renoprotective effects primarily through its antioxidant and free radical scavenging actions. The observed time-dependent attenuation of efficacy highlights the importance of ischemia duration as a critical determinant of therapeutic outcomes. Further studies are warranted to elucidate the molecular pathways underlying silymarin's renoprotective mechanisms and to evaluate its potential role in clinical settings involving transient renal ischemia.

Limitations

Although silymarin demonstrated significant protective effects against ischemia-reperfusion injury in this experimental model, the exact molecular pathways involved were not fully elucidated. Moreover, different doses and administration timings were not comparatively evaluated, which may influence the therapeutic efficacy. Future studies should explore dose-response relationships and underlying signaling pathways in greater detail.

Conclusions

In conclusion, the present study demonstrated that silymarin exerts significant renoprotective effects against renal ischemia–reperfusion injury in rats. These effects are mainly mediated through the enhancement of antioxidant defense mechanisms and the suppression of oxidative stress, as reflected by increased TAS and SOD activities and decreased TOS and MDA levels. Histopathological improvements further support the biochemical findings, particularly in the SIL45 group, where renal architecture was largely preserved. However, the protective efficacy of silymarin diminished with longer ischemia durations, indicating a time-dependent limitation in its renoprotective potential. Further experimental and clinical studies are warranted to elucidate the underlying molecular mechanisms and to explore the potential clinical application of silymarin in renal ischemic conditions.

Conflict of interest: The authors report no conflict of interest.

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Ethical approval: This experimental study was approved by the Animal Ethics Committee of Kahramanmaras Sutcu Imam University (approval no.: 2014/01-11) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH).

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Author contribution

Research concept and design: NAK, TS

Data analysis and interpretation: NAK, AYB

Data collection and/or assembly of data: TS, NAK, AYB

Writing the article: NAK, TS

Critical revision of the article: SR, AYB

Final approval of the article: SR, TS

All authors read and approved the final version of the manuscript.

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