ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(5): 4128–4134
doi: 10.25083/rbl/28.5/4128.4134

Received for publication, December, 10, 2023
Accepted, March, 10, 2024

Original article

The protective effects of glutathione plus silymarin on hepatic ischemia-reperfusion injuries produced in the kidney and lung tissues

DİLARA ALAKBARLI[1]*, KAĞAN KARABULUT[2], SEDA GÜN[3], RAMAZAN AMANVERMEZ[1]

[1] Department of Medical Biochemistry, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye.
[2] Department of General Surgery, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye.
[3] Department of Medical Pathology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye.

Abstract

Here we examine the efficacy of pretreatment with glutathione (GSH) plus silymarin (SM) on kidney and lung injury as a distant organ after hepatic ischemia reperfusion (IR). Rats were randomly separated into five groups: Sham, IR, GSH-IR, SM-IR, and SM plus GSH-IR. The treatment groups took 100 mg/kg of GSH, SM, or a combination of GSH plus SM 60 minutes prior to IR. The groups excluding sham were exposed to 30 minutes of ischemia and 24 hours of reperfusion. The rats were euthanized after 24 hours; blood, kidney, and lung specimens were gathered to perform analyses and pathology studies. As a result, serum creatinine and blood urea nitrogen (BUN) were significantly elevated in the IR group compared to sham. GSH administration prior to hepatic IR statistically declined IR-induced elevations of creatinine and BUN; likewise, creatinine and BUN were lower by an average of 19.8% and 54% in the SM plus GSH-IR group compared to the IR group, respectively. GSH, SM, and SM plus GSH pretreatments significantly reduced the kidney histopathological damage. Lung histopathologic damage scores on hepatic IR-induced lung injury were higher in the IR group than in the sham group, but lung pathological damage scores in the SM plus GSH-IR group were statistically low according to the IR group. Application of GSH plus SM before liver IR may be a potential therapy to mitigate remote injury of the kidney and lung resulting from hepatic IR.

Keywords Glutathione, silymarin, hepatic ischemia reperfusion, kidney injury, lung injury

*Corresponding author: Dr. Dilara Alakbarlı, Department of Medical Biochemistry, Faculty of Medicine, Ondokuz Mayıs University, TR-55200, Samsun / TÜRKİYE, email: dr_aliyeva@hotmail.com

Introduction

Hepatic IR injury is a serious complication in liver surgical interventions such as liver transplantation, major liver resections, and trauma. Hepatic IR cannot only result in acute liver damage, but also constantly leads to other organ injuries, including kidneys, lungs, myocardium, pancreas, and intestine, etc. Additionally, acute hepatic damage is induced by liver IR, which is known to result in an inflammatory response, oxidative stress, and marked endothelial cell apoptosis in the kidney, and activation of renin-angiotensin-aldosterone that can lead to acute kidney injury (AKI) [1, 2]. AKI or renal dysfunction after hepatic surgery and transplantation is highly common in clinical practice, as AKI is generally ascribed to renal ischemia due to hemodynamic instability in the perioperative period. Remote effects of liver IR are most frequently observed in the lungs and may lead to the improvement of acute lung injury (ALI) as well. Palladini et al. [3] and Colletti et al. [4] have also reported that hepatic IR injury is concerned with lung dysfunction in connection with neutrophil infiltration, edema, intra-alveolar hemorrhage, and tissue matrix metalloproteinase activation. The pathophysiology of hepatic IR-induced lung injury frequently resembles hepatopulmonary syndrome, which is a common trouble that has happened in patients subsequent to major liver surgery and transplantation associated with a systemic inflammatory response [5]. The occurrence of AKI and ALI after hepatic IR raises patient morbidity and mortality following the postoperative period, even affecting the efficacy of surgical operations and the long-term lifetime quality of patients [1-5]. In spite of calling attention to this prevalent clinical condition, AKI and ALI remain as diagnostic and therapeutic discomforts after liver IR. Therefore, a new therapeutic strategy can decrease the severity or incidence of AKI and ALI as a result of hepatic IR.

Silymarin, which possesses many therapeutic activities, including antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory, antibacterial, antiviral, antithrombotic, and vasodilatory properties, etc., is a complex mixture of polyphenolic molecules [6, 7]. The reduced form of glutathione is a thiol-containing tripeptide that works as a significant endogenous antioxidant against oxidative stress status, and its depletion in tissues immediately correlates to IR injury [8]. However, the effects of GSH plus SM therapy on hepatic IR-induced lung and kidney injury remain exactly unknown. Thus, we conducted a study to examine the efficacy of pretreatment with GSH plus SM on hepatic IR-induced kidney and lung injury in rats and to inspect the underlying mechanisms.

Materials and methods

The research procedure was approved by the animal experiments local ethics committee at Ondokuz Mayıs University (ethics no: 3, 26 Oct 2016). Fifty Sprague-Dawley male rats (300-350 g) were permitted to adapt to the local environment for seven days before being used. All animals were housed at 23±1 °C and 40-50% humidity under a 12-h light/dark cycle with free access to food and water. The rats were subjected to surgical processing after an overnight fast.

Study design

The rats were separated into 5 groups (10 for each group) as follows: Sham, IR, GSH-IR, SM-IR, and SM plus GSH-IR. Rats were anesthetized with an intraperitoneal application of 80 mg/kg ketamin (Ketalar®, Phizer) and 3 mg/kg xylazine (Rompun®, Bayer). Then, using aseptic techniques and materials, a midline laparotomy was made under anesthesia on rats. All anatomical structures in the portal triad (portal vein, hepatic artery, and bile duct) to the median and left liver lobes were exposed and subsequently blocked by using a non-traumatic microvascular clamp in order to create hepatic ischemia for 30 min. The clamp was carefully extracted after 30 min, and the rat's abdomen was directly closed with a permanent 4-0 silk suture, followed by a 24-hour reperfusion time. An equal volume of normal saline (NS) solution containing 10% of DMSO was intraperitoneally applied to replace the drug solution under anesthesia in the sham group, and then rats were exposed to laparotomy as well as exposure to the portal triad anatomical structures without hepatic ischemia. Rats in the IR group were subjected to ischemia and reperfusion as defined above. Reduced form of glutathione (Sigma Co., GSH) and SM (Solgar Co., milk thistle standardized capsule) were separately dissolved in physiological saline and added with 10% DSMO. The SM-IR group took SM (100 mg/kg bwt.,i.p.); the GSH-IR group got GSH (100 mg/kg bwt., i.p.), and their mix (i.p.) was applied for the SM plus GSH-IR group, 60 min before hepatic IR. Note that the body temperature of the animals was kept close to 37 °C with the backing of a heating lamp. Afterwards, an injection of cefuroxime (an antibiotic, 20 mg/kg/day, i.m.) after surgical procedures was applied to all animals. At the end of a 24-hour period, the rats were anesthetized using xylazine and ketamine. The abdomens of rats were reopened for the collection of blood samples, kidney and lung specimens. Thereafter, blood was taken from the heart with an injector and collected in centrifuge tubes. The serum was allotted using a centrifuge device at 2.500 g and stocked at -80 °C for later analyses. Kidney and lung specimens were washed with NS and then fixed in a neutral formaldehyde solution.

Biochemical assessments

Serum values of creatinine and blood urea nitrogen (BUN) were estimated by an automated chemical analyzer (Cobas 6000 C501). Serum hsCRP (high sensitivity C-reactive protein) was measured by an automated analyzer (Beckman Coulter Immage 800). Measurements of serum hsp 70 and pro-apoptotic protein caspase 3 were estimated by using Elisa Kits for rats according to the instructions of the manufacturer.

Histopathological assessment of renal injury

The kidney tissues were fixed with 10% neutral formaldehyde, embedded in paraffin blocks, cut into 4-µm portions, and stained with hematoxylin and eosin (H&E). The renal tissue edges were inspected with an Olympus BX51 microscope (Olympus Optical Co., Japan) by a pathologist unknown to the study. The kidney pathological lesions in the experimental groups were evaluated according to the severity (score: 0-3) of proximal tubule simplification, peritubular leukocyte infiltration, renal cortical vacuolization, and proximal tubule hypereosinophilia as described by Park et al. [2].

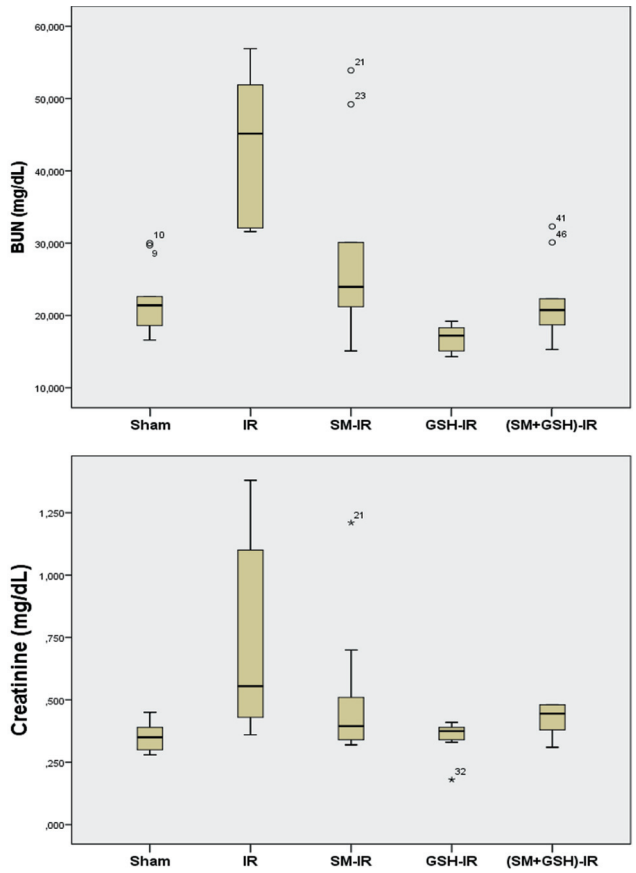

Fig. 1. Effects of pretreatment with SM and GSH on serum levels of BUN and creatinine after hepatic IR injury. GSH administered at 100 mg/kg protects against acute renal injury in GSH-IR group rats subjected to 30 minutes of liver ischemia and 24 hours of reperfusion.

Histopathological examinations of lung injuries

Pathological changes in the lung tissue were evaluated morphologically. The lung tissues were fixed with 10% neutral formaldehyde, embedded in paraffin blocks, cut into 4-µm portions, and stained with hematoxylin and eosin (H&E). An expert pathologist assessed and scored the degree of lung injury. The degree of microscopic injuries was graded on a scale of 0-3 (0, absent and appears normally; 1, mild; 2, moderate; 3, severely) for intra-alveolar hemorrhage, interstitial perivascular infiltration of neutrophils, interstitial congestion, and edema. In addition, a total lung damage score was assessed as the sum of four components.

Statistical analysis

IBM Statistics 22.0 software was used to analyze the data. All data were initially evaluated for the normality assumption and analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. To estimate the severity of the pathological lesions, the Kruskal-Wallis analysis of variance was utilized to detect the statistical importance of differences in the study groups. So, the Mann-Whitney U test with Benforroni's correction was applied for comparisons between the groups. Data are presented as median (min-max) for determined parameters. The statistical differences were set at $p<0.05$.

Results

The beneficial effects of SM and GSH against renal injury after liver IR

The hepatic IR group displayed renal dysfunction as compared to the sham group, as reflected by a significant rise in serum creatinine and BUN ($p<0.05$, Figure 1). Both BUN and creatinine were significantly decreased in the rats pretreated with GSH (100 mg/kg i.p.) prior to surgery ($p<0.001$, $p=0.004$). Moreover, serum levels of creatinine and BUN in rats treated with a combination of SM plus GSH prior to hepatic IR were lower by an average of 19.8% and 54%, respectively. However, no statistical difference was obtained for serum levels of hsCRP, hsp 70, and caspase-3 among the groups in the present hepatic IR rat model ($p>0.05$, Table 1).

Renal histopathological changes attenuated by SM and GSH pretreatment

The sham group showed normal renal glomerular and tubular structures (Figure 2A). However, in the IR group, histopathological changes in renal tissue sections were observed, including marked peritubular leukocyte infiltration, proximal tubule hypereosinophilia, and vacuolization in tubular epithelium, but no necrosis in the kidneys at 24 h following liver IR (Figure 2B). On the other hand, the severity

Table 1. Serum levels of hsCRP, hsp 70 and caspase 3 in the experimental groups.

Groups (n=10)	Parameters		
	hsCRP (mg/dl)	Hsp 70 (ng/ml)	Caspase 3 (ng/ml)
#Sham	0.142 (0.105-0.180)	111.75 (86.56-207.18)	0.95 (0.41-1.50)
#IR	0.160 (0.123-0.221)	139.36 (85.60-160.55)	1.32 (0.61-1.75)
#SM-IR	0.154 (0.113-0.201)	126.19 (67.14-140.74)	0.69 (0.29-1.67)
#GSH-IR	0.156 (0.131-0.194)	119.08 (95.94-169.56)	1.01 (0.81-1.22)
#SM plus GSH-IR	0.150 (0.106-0.210)	116.70 (84.81-165.96)	1.03 (0.59-1.42)

Data are expressed as median (min-max) for parameters.

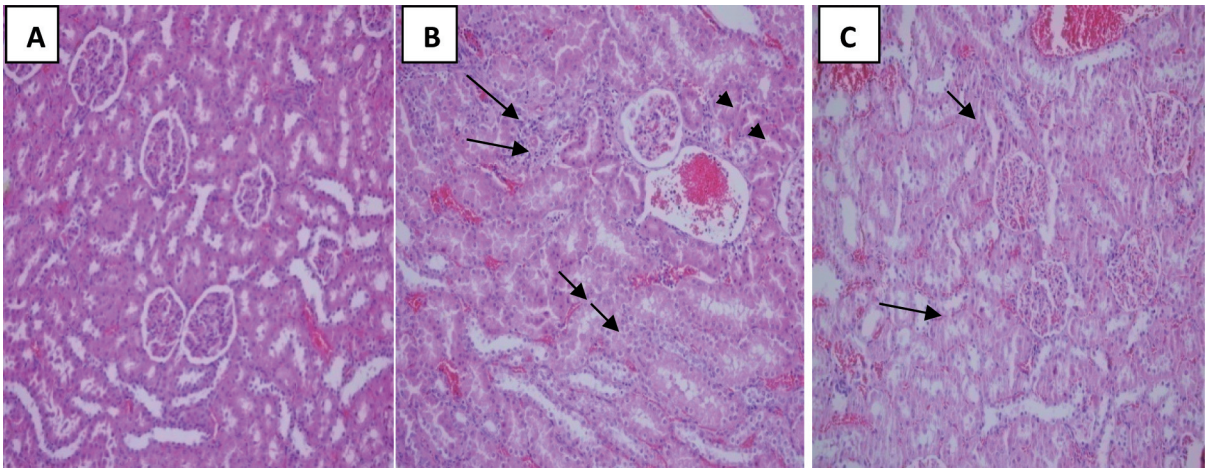

Fig. 2. Effects of SM and GSH pretreatment on histopathologic renal alterations in rats exposed to 30-min of hepatic ischemia and 24-h reperfusion. **A**. Normal renal glomerular and tubular structure in the sham-operated group, H&E X200. **B**. Marked vacuolization in tubular epithelium (short arrow), hypereosinophilia (arrowhead), and peritubular leukocyte infiltration (long arrow) in a renal tissue section of the IR group, H&E X200. **C**. Slight vacuolization in tubular epithelium (long arrow) and lightly hypereosinophilia (short arrow) in the renal tissue section treated with GSH prior to hepatic IR, H&E X200.

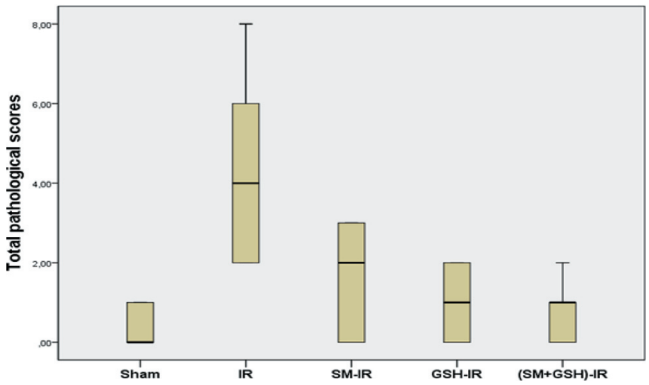

Fig. 3. The effects of SM and GSH on histopathologic renal damage scores after 30-min hepatic ischemia and 24-h reperfusion. Histopathologic renal damage scores of 2 (0-3) in the SM-IR, 1 (0-2) in the GSH-IR, and 1 (0-2) in the SM plus GSH-IR groups were statistically lower than in the IR group [4 (2-8)] in relation to AKI.

Table 2. Histopathological damage scores in the lung tissues of experimental groups.

Groups (n=10)	**Histopathological damage score**
#Sham	2.5 (0-4)
#IR	6.0 (4-9)a
#SM-IR	5.5 (4-8)
#GSH-IR	5.0 (3-8)
#SM plus GSH-IR	5.0 (3-8)b

Data are expressed as median (min-max) for damage score. a-b indicates statistically significant groups.

of renal lesions or degenerative alterations, including total pathological scores like vacuolization, peritubular leukocyte infiltration, proximal tubule hypereosinophilia, and proximal tubule simplification, was statistically reduced in GSH, SM, and SM plus GSH-treated groups when compared to the IR group, as shown in Figure 3 ($p=0.007$, $p=0.001$, $p<0.001$, respectively).

The histopathological changes of the lungs reduced by SM plus GSH pretreatment

The findings of lung histopathological damage scores on hepatic IR-induced lung injury were observed to be higher in the IR, GSH-IR, SM-IR, and SM plus GSH-IR groups than in the sham group ($p<0.001$), but the lung histopathological damage score in the SM plus GSH-IR group pretreated with a mixture of SM plus GSH was statistically low with respect to the IR group at the end of 24-h, as shown in Figure 4 and Table 2 ($p<0.05$).

Discussion

The results of the present study demonstrated that AKI and ALI induced by hepatic IR were confirmed by the raised serum levels of BUN and creatinine and the increased renal and lung histopathological damage scores in the IR group when compared to sham. In addition, GSH plus SM treat-

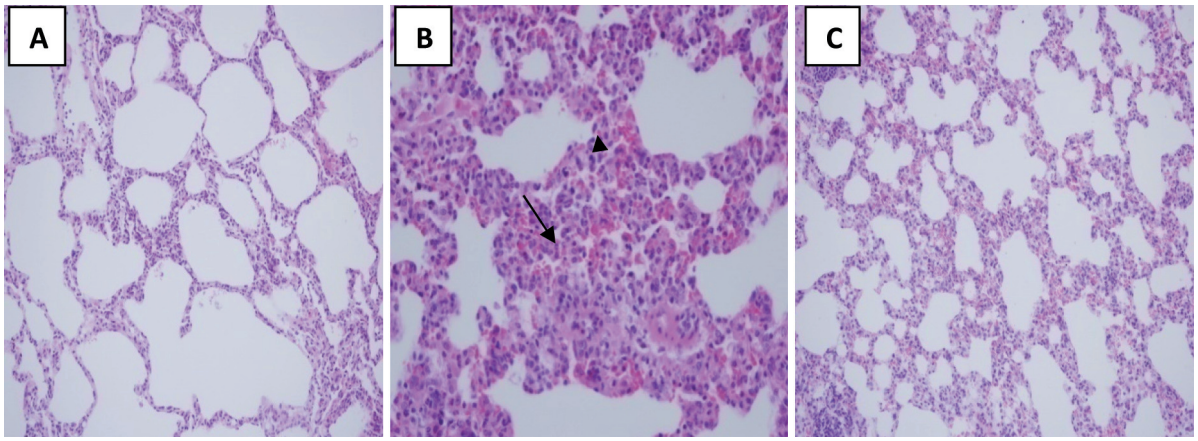

Fig. 4. Lung histopathology after 30-min hepatic ischemia and 24-h reperfusion. (**A-C**) Representative images of lung sections stained with H&E. In the (**A**) sham group: Mild neutrophil infiltration, mild interstitial congestion, and edema on lung section (HEX200); (**B**): Moderate-severe leukocyte infiltration (arrow), moderate alveolar interstitial congestion (arrowhead), and edema on lung section (HEX400) in the IR group; (**C**): Mild-moderate leukocyte infiltration, mildly alveolar interstitial congestion, and edema on lung section (HEX200) in the SM plus GSH-IR group.

ment before hepatic IR significantly declined levels of BUN and creatinine and the lung and renal histopathologic damage scores on hepatic injury caused by IR (Figures 1 and 3, Table 2).

30-min hepatic ischemia followed by 24-h reperfusion resulted in a significant elevation of serum creatinine and BUN levels, according to our results, representing that renal function was impaired. The findings of the current study agree with the previous findings of Gonul et al. [9] and Lee et al. [10] who reported induction of renal and hepatic damage by liver IR injury. A number of scientific reports have indicated that acute liver damage caused by hepatic IR is known to evoke a systemic inflammatory response, humoral mediators (cytokines, chemokines, prostaglandins, and stress hormones), glutathione depletion, and oxidative stress that result in damage and impaired function of distant organ systems [1, 11-13]. For instance, hepatic IR injury has been shown to be associated with a significant rise in the expression of proinflammatory mediators and oxidative stress markers in the kidney, causing a decline in endogenous antioxidants such as GSH in plasma, hepatic, and renal tissues [4, 14-17]. In the present study, serum levels of creatinine and BUN were significantly elevated after liver IR, and the GSH treatment decreased this increase. Also, renal histopathological damage scores were lower in relation to GSH supplementation (Figures 1 and 3), suggesting that GSH might contribute to the reduction in the degree of AKI after liver IR. Restoration of the GSG/GSSH ratio through GSH therapy prior to surgery suppresses an oxidative stress condition, apoptosis, and the inflammatory process (TNF-α-mediated) augmented by hepatic IR injury [16]. Moreover, silymarin treatment (10 mg/100g bwt.) resulted in significant hepato-renal protection during CCL_4-induced oxidative stress by restoring liver and kidney function tests [18]. The latest research reports have shown that silymarin treatment can reduce serum levels of BUN and creatinine involved in necrosis of renal tubular cells and tubular damage and then improve kidney function [19, 21]. In our study, serum BUN values and kidney histopathologic damage findings were statistically decreased in rats pretreated with solely SM or SM plus GSH prior to liver IR. As a result of these findings, administration of GSH plus SM prior to liver surgery may prevent kidney injury induced by hepatic IR.

Hepatic IR injury and hemodynamic perturbations, initially located in the liver but targeting subsidiary lungs, could be involved in several pathologies or postoperative pulmonary complications that might frequently form pulmonary edema, pleural effusion, acute respiratory distress syndrome (ARDS), atelectasis, and pneumonia in patients after hepatectomy [22-24]. In our study, as presented in Table 2 and Figure 4, pathological changes (intra-alveolar hemorrhage, leukocyte infiltration, interstitial congestion, and pulmonary edema) in lung parenchyma were observed in greater quantity in the IR group than in the group that received SM plus GSH prior to liver IR, considering that pretreatment with SM plus GSH may protect against lung injury after hepatic IR damage. Intravenous infusion of GSH has been shown to effectively attenuate vascular oxidative stress after IR [25]. A previous study reported that pretreatment with SM (at a dose of 100 or 200 mg/kg, i.p.) reduced the high histopathological changes of the lungs in an LPS-induced ARDS rat model, improving pulmonary function and lung tissue damage [26]. Based on the above findings, application of GSH plus SM prior to liver IR seems to have decreased the lung parenchymal lesions in hepatic IR-induced ALI.

Caspase-3, which is considered to be a specific indicator of apoptosis, hsCRP, which is a biomarker of inflammation, and hsp70, which is usually elevated in subjects with inflammation, appear to be higher in the IR group as compared to the sham group, but no significant differences were obtained for serum levels of these analyses between the IR group and pretreatment groups (Table 1). It is limited due to using only one dose of these agents, and further investigation of alternative doses is needed to confirm the current results in relation to the estimation of oxidative stress markers, inflammatory mediators, and apoptosis-related indicators in the kidney and lung tissues for hepatic IR-induced AKI and ALI.

In conclusion, pretreatment with GSH plus SM exhibits the therapeutic effects on AKI and ALI induced by hepatic IR. Administration of GSH plus SM before liver IR can effectively protect the liver [27], but SM used in combination with GSH could also restore impaired kidney function and alleviate the renal and lung histopathological lesions caused by hepatic IR injury. However, further studies may be required to ratify these results.

Conflict of Interest

The authors of this study declare no conflicts of interest.

Financial Disclosure

The authors of this study disclose no financial, commercial, or personal relationships with other people or organizations.

Ethical statement

This research was approved by the animal experiments local ethics committee at Ondokuz Mayıs University (ethics no: 3, 26 Oct 2016).

References

1. Nastos C, Kalimeris K, Papoutsidakis N, Tasoulis MK, Lykoudis PM, Theodoraki K, et al.Global consequences of liver ischemia reperfusion injury. Oxidative Medicine Cellular Longevity. 2014: DOI: 10.1155/2014/906965. PMID: 24799983.
2. Park SW, Chen SWC, Kim M, Agati VD, Lee HT. Human active protein C attenuates both hepatic and renal injury caused by hepatic ischemia and reperfusion injury in mice. Kidney International. 2009; 76: 739-750.
3. Palladini G, Ferrigno A, Rizzo V, Tarantola E, Bertone V, Freitas I, et al. Lung matrix metalloproteinase activation following partial hepatic ischemia/reperfusion injury in rats. Scientific Word Journal. 2014: DOI: 10.1155/2014/867548. PMID: 24592193.
4. Colletti LM, Kunkel SL, Walz A, Burdick MD, Kunkel RG, Wilke CA, et al. Chemokine expression during hepatic ischemia/reperfusion-induced lung injury in the rat. The Journal of Clinical Investigation. 1995; 95(1): 134-141.
5. Yeh DYW, Yang YC, Wang JJ. Hepatic warm ischemia-reperfusion-induced increase increase in pulmonary capillary filtration is ameliorated by administration of a multidrug resistance-associated protein 1 inhibitor and leukotriene D4 antogonist (MK-571) through reducing neutrophil infiltration and pulmonary inflammation and oxidative stress in rats. Transplantation Proceedings. 2015; 47(4):1087-1091.
6. Akbari-Kordkheyli V, Abbaszadeh-Goudarzi K, Nejati-Laskokalayeh M, Zarpou S, Khonaktar-Tarsi A. The protective effects of silymarin on ischemia-reperfusion injuries: A mechanistic review. Iranian Journal of Basic Medical Sciences. 2019; 22: 968-976.
7. Sangdehi SRM, Moghaddam AH, Ranjbar M. Anti-apoptotic effect of silymarin-loaded chitosan nanoparticles on hippocampal caspase-3 and Bcl-2 expression following cerebral ischemia/reperfusion injury. International Journal of Neuroscience. 2022; 132(11): 1102- 1109.
8. Arrivi A, Barilla F, Carnevale R, et al. Protective biomolecular mechanisms of glutathione sodium salt in ischemia-reperfusion injury in patients with acute coronary syndrome-ST-elevation myocardial infarction. Cells. 2022; 11(24): e3964.
9. Gonul Y, Kazandı S, Kocak A, Ahsen A, Bal A, Karavelioglu A, et al. Interleukin-18 binding protein pretreatment attenuates kidney injury induced by hepatic ischemia reperfusion. The American Journal of Medical Science. 2016; 352(2): 200- 206.
10. Lee HT, Park SW, Kim M, D'Agati VD. Acute kidney injury after hepatic ischemia reperfusion injury in mice. Laboratory Investigation. 2009; 89(2): 196-208.
11. Kimura F, Shimizu H, Yoshidome H, Ohtsuka M, Kato A, Yoshitomi H, et al. Circulating cytokines, chemokines, and stress hormones are increased in patients with organ dysfunction following liver resection. Journal of Surgical Research. 2006; 133(2): 102-112.
12. Kadkhodaee M, Mikaeili S, Zahmatkesh M, Golab F, Seifi B, Arab HA, et al. Alteration of renal functional, oxidative stress and inflammatory indices following hepatic ischemia-reperfusion. General Physiology Biophysics. 2012; 31(2): 195-202.
13. Kudoh K, Uchinami H, Yoshioka M, Seki E, Yamamoto Y. Nrf2 activation protects the liver from ischemia/

reperfusion injury in mice. Annals of Surgery. 2014; 260(1): 118-127.
14. Seifi B, Kadkhodaee M, Delavari F, Mikaeili S, Shams S, Ostad SN. Pretreatment with pentoxifylline and N-acethylcysteine in liver ischemia reperfusion-induced renal injury. Renal Failure. 2012; 34(5): 610-615.
15. Jochmans I, Meurisse N, Neyrinck A, Verhaegen M, Monbaliu D, Pirenne J. Hepatic ischemia/reperfusion injury associates with acute kidney injury in liver transplantation: Prospective chort study. Liver Transplantation. 2017; 23: 634-644.
16. Suyavaran A, Ramamurthy C, Mareeswaran R, Subastri A, Rao PL, Thirunavukkarasu C. TNF-α suppression by glutathione preconditioning attenuates hepatic ischemia reperfusion injury in young and aged rats. Inflammation Research. 2015; 64(1): 71-81.
17. Polat C, Tokyol C, Kahraman A, Sabuncuoglu B, Yılmaz S. The effects of desferrioxamine and quercetin on hepatic ischemia-reperfusion induced renal disturbance. Prostaglandins Leukotrienes and Essential Fatty Acids. 2006; 74(6): 379-383.
18. El-Maddawy ZK, Gad SB. Hepato-renal protection of silymarin in comparison with vitamin E in rats. Global Journal of Pharmacology. 2012; 6(3): 236-244.
19. Dabak DO, Kocaman N. Effects of silymarin on methotrexate induced nephrotoxicity in rats. Renal Failure. 2015; 37(4): 734-739.
20. Bektur NE, Sahin E, Baycu C, Unver G. Protective effects of silymarin against acetaminophen-induced hepatotoxicity and nephrotoxicity in mice. Toxicology and Industrial Health. 2016; 32(4): 589-600.
21. Demir N, Basunlu MT. Comparative nephroprotective effects of silymarin, acetylcysteine, and thymoquinone against carbon tetrachloride-induced nephrotoxicity in rats. Iranian Red Crescent Medical Journal. 2017; 19(1): DOI: 10.5812/ircmj.37746.
22. Feltracco P, Carollo C, Barbieri S, Pettenuzzo T, Ori C. Early respiratory complications after liver transplantation. World Journal of Gastroenterology. 2013; 19(48): 9271-9281.
23. Lepere V, Vanier A, Loncar Y, Lemoine L, Vaillant JC, Monsel a, et al. Risk factors for pulmonary complications after hepatic resection: role of intraoperative hemodynamic instability and hepatic ischemia. BMC Anesthesiology. 2017; 17(1): 84.
24. Loncar Y, Tartrat N, Lastennet D, Lemoine L, Vaillant JC, Savier E, et al. Pulmonary infection after hepatic resection: associated factors and impact on outcomes. Clin Res Hepatol Gastroenterol. 2022; 46(4): DOI: 10.1016/j.clinre.2021.10733. PMID: 34146724.
25. Jaeschke H, Woolbright BL. Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species. Transplantation Reviews. 2012; 26(2): 113- 114.
26. Zhu Z, Sun G. Silymarin mitigates lung impairments in a rat model of acute respiratory distress syndrome. Inflammopharmacology. 2018; 26(3): 747-754.
27. Aliyeva D, Amanvermez R, Karabulut K, Gun S. The effects of silymarin plus glutathione on the prevention of liver ischemia-reperfusion injury. Brazilian Journal of Pharmaceutical Sciences. 2022; 58: DOI: 10.1590/s2175-97902022e20561.