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Silymarin protects against sepsis-induced acute liver and kidney injury via anti-inflammatory and antioxidant mechanisms in the rat

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Abstract

Sepsis is a leading cause of death among intensive care patients. During sepsis, exaggerated reaction to infection leads to massive production of reactive oxygen species and inflammatory mediators, which eventually leads to multiple organ damage. Silymarin is a well-known antioxidant and cytoprotective agent, which showed protective effects in different models of disease. Thus, we hypothesized that silymarin would be protective against sepsis-induced liver and kidney injury. Sepsis was induced in rats by the cecal ligation and puncture (CLP) method. Rats were divided into sham, CLP-non-treated and CLP treated with silymarin (100 mg/kg, i.p. 1 h following CLP). After 24 h, rats were euthanized for blood and tissue samples, which were used for assessment of MDA, NO, GSH, IL-6 and TNF-α levels and SOD activity, in addition to renal and hepatic function parameters. Survival study was conducted using another set of animals following the same previously mentioned procedure. Silymarin showed protective effects evidenced by enhanced overall survival following sepsis (80% in silymarin-treated vs. 20% in septic group), in addition to improvement of hepatic and renal function parameters and reduction of MDA, NO, IL-6 and TNF-α levels. Moreover, silymarin supported the endogenous antioxidant mechanisms via elevation of GSH levels and reinforcement of SOD activity. In conclusion, silymarin protects against sepsis-induced hepatic and renal injury, possibly via antioxidant and anti-inflammatory mechanisms.

1. Introduction

Sepsis is defined as a ''life-threatening organ dysfunction caused by dysregulated host response to infection'' [1, 2]. With a global incidence rate of 30 million cases/year and a death toll of 6 million, sepsis is indeed a leading cause of death in intensive care units [3]. The pathophysiology of sepsis involves two distinct phases; early and late. The early phase is mainly inflammatory in nature characterized by what is known as cytokine storm. The hallmark of this phase is the massive release of the inflammatory cytokines such as TNF-α and IL-6 [2, 4-6]. In addition, because of the exaggerated inflammatory signaling, this early phase is also characterized by profound production of reactive oxygen and nitrogen species such as superoxide radical and nitric oxide [4, 6-8]. As the late phase of sepsis develops, tissue ischemia occurs due to either systemic or local imbalance between oxygen delivery and tissue demand [9], which proceeds to tissue hypoxia, mitochondrial dysfunction, and apoptosis. These pathologic mechanisms are thought to be the major determinants of multiple organ failure during sepsis [10, 11]. Thus, these pathways are important targets to improve survival and prevent organ damage in septic patients. Indeed, several studies established the protective effect of antioxidants against sepsis-induced organ injury [7, 12-14].

The liver and the kidney are among the most vulnerable and early affected organs during sepsis [15, 16]. Sepsis-induced liver dysfunction is considered a contributing factor to multiple organ dysfunction and sepsis-induced death. Being a lymphoid organ, the liver plays a pivotal role in clearing bacteria and its toxins, however, these actions extend during sepsis to encompass modulation of the inflammatory response, immunosuppression, and organ damage [16]. For example, Kupffer cells, which play a critical role in clearing bacterial products, are responsible for producing inflammatory cytokines such as TNF-α, IL-6, and interferon-gamma (IFN-γ) in the early response phase, as well as the production of reactive oxygen species and NO. These mediators will eventually lead to the damage of Kupffer cells themselves, and reduced efficiency of bacterial products removal. Leakage of such products increases tissue neutrophil infiltration. Thus, the injured liver can induce a severe systemic harmful inflammatory response in other organs during sepsis [17]. On the other hand, the endotoxemia-induced acute kidney injury is associated with hemodynamic and pro-inflammatory alarms. Moreover, a functional kidney is crucial to counteract sepsis-induced generalized systemic vasodilatation, which is strongly NO-dependent [18]. Thus, deterioration of renal function during sepsis contributes to the overall mortality and morbidity [15, 19, 20].

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Silymarin is a naturally occurring herbal product extracted from the seeds and fruits of *Silybum marianum*, and has been long used to treat liver conditions [21, 22]. Like other flavonoids, silymarin exhibits various pharmacological and therapeutic applications due to its antioxidant and anti-inflammatory properties [23-25]. Thus, based on the potent antioxidant and anti-inflammatory effects of silymarin, we hypothesized that silymarin would improve the survival of septic rats and exert a protective effect against sepsis-induced liver and kidney injury.

2. Materials and methods

2.1. Animals

Female Wistar rats (180-200 g) were purchased from Nahda University at Beni-Suef (NUB, Beni-Suef, Egypt). Animals were kept for a 2-week acclimatization period housed at 24 °C under a 12 h light-dark cycle with free access to food and water before the day of experiment. All experiments were approved by the Commission on Ethics of Scientific Research for the Ethical Principles and Guidelines of the Care and Use of Laboratory Animals, Faculty of Pharmacy, Minia University (Code number of the project: 37/2019)

2.2. Experimental design

This study involved two separate arms: mechanistic and survival studies. For the mechanistic study, 18 female Wistar rats were randomly divided into three groups (n = 6): sham-operated, septic non-treated group (CLP; received equivalent volumes of saline), and septic group treated with silymarin (100 mg/kg, i.p., 1 h after the induction of sepsis) [26]. Rats in this design were killed 24 hours after induction of sepsis. For the survival study: the same set of groups were employed (n = 10 for each), but all groups were observed for survival for 10 days.

2.3. Induction of Sepsis by cecal ligation and puncture

The CLP was adopted as a model of sepsis as previously described [12, 27]. Rats were anesthetized by ketamine-xylazine combination (50 and 10 mg/kg, respectively) [12] and a ventral incision was made in the lower left quadrant of the abdomen after shaving and disinfecting the abdominal wall. The cecum was then exposed and ligated with a 3/0 silk surgical suture, and then two through-and-through punctures were done in the ligated part using an 18-gauge syringe needle. To limit the variability in the severity of sepsis, a fixed proportion (75%) of the cecum length was subjected to ligation throughout the study. The ligated cecum was squeezed gently and returned into the abdominal cavity. At the end of the procedure the incision was sutured and normal saline in a dose of 30 ml/kg was subcutaneously injected for resuscitation. Sham-operated rats were exposed to the same operating steps and resuscitation without CLP.

2.4. Tissue isolation and preparation

Twenty-four hours after sepsis induction, the animals were anaesthetized by sodium pentobarbital (50 mg/kg). Blood samples were collected via cardiac puncture then animals were euthanized by decapitation. Blood was left at room temperature to coagulate. The collection of sera was carried out after centrifugation for 10 minutes at 15000 rpm using a benchtop centrifuge (Centurion Scientific Limited). The liver and kidneys were cautiously dissected. For biochemical analyses, portions of the removed tissues were flash-frozen in liquid nitrogen then kept at -20°C till the time of assay. Other portions of tissues were kept in buffered 10% formalin solution for histopathological studies. At the time of biochemical measurements, tissue homogenates were prepared as 10 % w/v in phosphate-buffered saline using a hand-held tissue homogenizer (Cole-Parmer LabGen 7) followed by centrifugation at 15000 rpm for 10 minutes using a cooling centrifuge (Scilogex) to collect clear supernatants.

2.5. Assessment of liver function parameters

The serum levels of ALT (EC2.6.1.2) and AST (EC2.6.1.1) were determined by colorimetric assay kits purchased from Diamond Diagnostics, Egypt, according to the manufacturer's instructions.

2.6. Assessment of kidney function parameters

Serum creatinine was determined by a colorimetric assay using a commercially available kit (Cat. No. CR 12 50, Biodiagnostic, Egypt) and serum level of cystatin-c (cys-c) was determined by a Sandwich-ELISA kit (Cat. No. E-EL-R0304, Elabscience Biotechnology Inc., Egypt) according to the instructions provided by the manufacturer.

2.7. Assessment of serum inflammatory cytokines

Cytokine profile was determined in serum samples using Sandwich-ELISA kits for TNF-α (Cat. No. E-EL-R0019) and the serum IL-6 (Cat. No. E-EL-R0015; Elabscience Biotechnology Inc., Egypt).

2.8. Assessment of liver and kidney oxidative stress markers

Tissue thiobarbituric acid reactive species (TBARs) was measured in liver and kidney homogenates as a marker of lipid peroxidation using a commercially available kit (Cat. No. MD 25 29, Biodiagnostic, Egypt), and levels of nitrite were assayed by a commercially available kit (Cat. No. NO 25 33, Biodiagnostic, Egypt) applying the assay procedures provided by the supplier.

2.9. Assessment of liver and kidney antioxidant defense activity

Superoxide dismutase SOD activity was determined spectrophotometrically using a commercial kit (Cat. No. SD 25 21, Biodiagnostic, Egypt) while the concentration of reduced-glutathione GSH in liver and kidney homogenates was assessed using a colorimetric assay (Cat. No. GR 25 11, Biodiagnostic, Egypt) according to the manufacturer's recommendations.

2.10. Histopathological examination

Liver and kidney tissues were processed after fixation in neutral buffered formalin solution (10%) and Hematoxylin and Eosin (H&E) staining using standard techniques [28]. Sections were analyzed and pictures were digitally taken using a high-resolution digital camera mounted on the microscope (Olympus, Tokyo, Japan).

2.11. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) and were analyzed for statistically significant differences using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer post-analysis test. Results were considered significant at p-value < 0.05. GraphPad Prism® 6 software (San Diego California, USA) was used for statistical calculations. Results of the survival study were analyzed for statistically significant differences using the log-rank (Mantel-Cox) test to detect intergroup differences.

3. Results

3.1. Effect of silymarin on CLP-induced mortality

As shown in Figure 1, 30% of the rats in the CLP group died within the first 24 h after surgery, while no rats died in the sham-operated control group for the whole period of observation (10 days). Moreover, the untreated CLP group mortality reached 70% in the second day. On the other hand, the silymarin-treated CLP group showed no mortality on the first day after surgery and only 20% in the first two days. In addition, silymarin treatment showed a significant (p<0.05) improvement in the overall survival (80%) when compared to the untreated CLP group (20%).
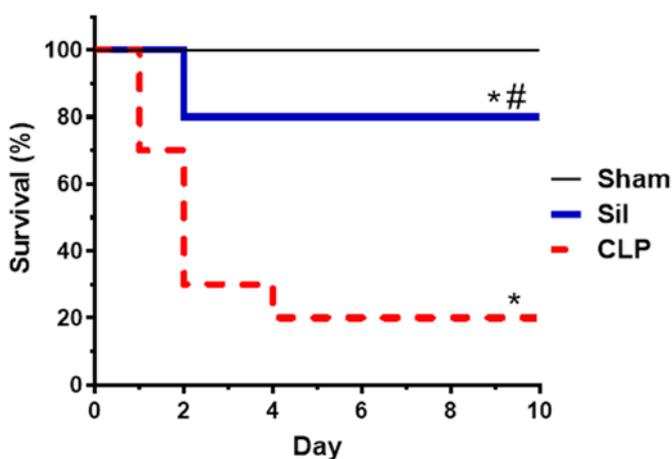
Figure 1: Effect of Silymarin (100 mg/kg) on CLP-induced mortality. Animals were monitored daily for 10 days. Data are presented as the survival percentage of animals (n=10 rats per group). *Significantly different compared to sham group at *(p<0.05)*, #significantly different compared to CLP group at *(p<0.05)*. Sil = Silymarin, CLP = cecal ligation and puncture.

3.2. Effect of silymarin on CLP-induced liver injury

The serum liver function parameters ALT and AST were markedly elevated in septic rats compared to sham animals (105.5 ± 14.1 vs 17.9 ± 1.5 U/L, and 33.9 ± 3.0 vs 16.2 ± 1.3 U/L, respectively) as shown in Figure 2A and 2B. On the other hand, administration of silymarin inhibited sepsis-induced increases in ALT and AST (23.6 ± 3.0 and 22.5 ± 2.2 U/L, respectively). Histopathological examination of liver tissues revealed normal lobular architecture in sham-operated rats. Hepatocytes were arranged in cords characterized by radial arrangement around the central vein (CV). In contrast, samples from the untreated CLP group showed numerous degenerative changes in hepatocytes either with foamy vacuolated cytoplasm and deformed nuclei or apoptotic cells with acidophilic cytoplasm and dark small nuclei. Moreover, sinusoidal dilatation and congestion, areas of hepatocyte atrophy, and proliferation of bile ducts were clearly observed in the untreated CLP group. These findings were significantly reduced in the silymarin-treated CLP animals, which showed preserved general lobular architecture and no evidence of major morphological injury contrary to the untreated CLP group. However, only few apoptotic cells were observed in the silymarin-treated CLP group (Figure 2C).

3.3. Effect of silymarin on CLP-induced kidney injury

Both creatinine and cystatin-c were markedly increased in the sera of untreated septic rats in comparison with sham-operated animals. On the other hand, administration of silymarin 1-h after induction of sepsis by CLP abrogated this increase (Figure 3A and 3B). Normal histological structure of the renal cortex in the sham control group was observed demonstrating normal renal corpuscles as well as proximal and distal convoluted tubules. In contrast, the untreated CLP group showed disturbed cortical architecture with marked distortion of renal tubules. Tubular epithelium of the untreated CLP group showed signs of apoptosis, vacuolated cells and cellular debris in the lumen. In addition, these sections showed vascular congestion, interstitial hemorrhage and distorted corpuscles with widening of Bowman's space. On the other hand, silymarin treatment protected the kidneys of septic rats, where the sections showed preserved normal histological architecture (Figure 3C).

3.4. Effect of silymarin on serum TNF-α and IL-6 of septic rats

The serum level of TNF-α was significantly elevated in the CLP-induced septic animals when compared to the sham-operated controls. Similarly, the serum level of IL-6 was significantly increased in septic rats when compared to their sham controls. On the other hand, administration of silymarin 1 h after induction of sepsis by the CLP procedure resulted in significant reduction of sepsis-induced elevation of serum TNF-α and IL-6 (Figure 4).
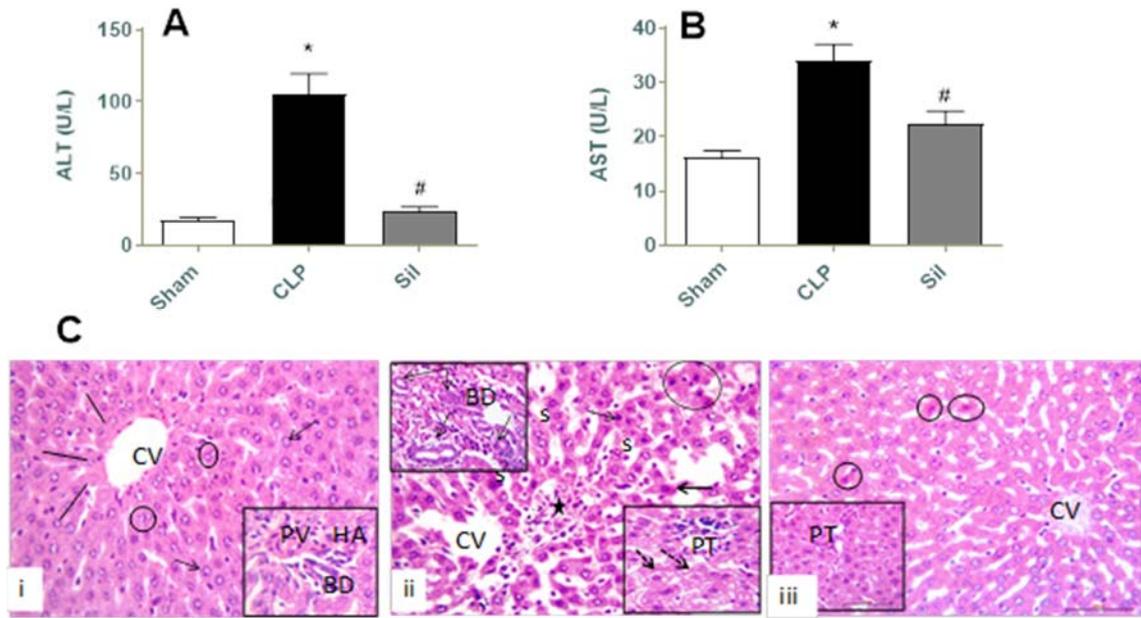
Figure 2: Effect of Silymarin treatment (100 mg/kg) on the liver of septic rats. Effect of Silymarin on serum ALT (**A**) and AST (**B**). Sil = Silymarin-treated, CLP = cecal ligation and puncture. Data were analyzed with one-way ANOVA followed by Tukey–Kramer test for multiple comparisons. *Significantly different from sham-operated rats ($p<0.05$); #Significantly different from untreated CLP-group ($p<0.05$). **C**: Representative photomicrographs of rat liver tissues (H&E x400) from the sham control group (i) showing normal lobular architecture of polygonal hepatocytes arranged in plates with characteristic rounded vesicular nuclei (arrows) radiating from the central veins (CV) and normal sinusoidal spaces (S). Inset: portal tract containing branches of the portal vein, hepatic artery (HA), and bile duct (D). Notice binucleated hepatocytes (circle). The untreated CLP-group (ii) showing disturbed lobular architecture surrounding CV, necrotic foci (star), sinusoidal dilatation (S) and apoptotic hepatocytes with dark small nuclei either in clusters (circle) or scattered (arrows). Numerous hepatocytes with foamy vacuolated cytoplasm (dashed arrows) around PT (right inset). Notice proliferation of bile ducts (arrows in left inset). The Silymarin-treated CLP group (iii) showing restored normal lobular architecture with apparently normal hepatocytes (arrows) radiating from slightly congested central vein (CV) and slightly dilated sinusoids (S). Notice few scattered vacuolated cells around PT (arrows in inset) or few scattered apoptotic cells around CV (circles).
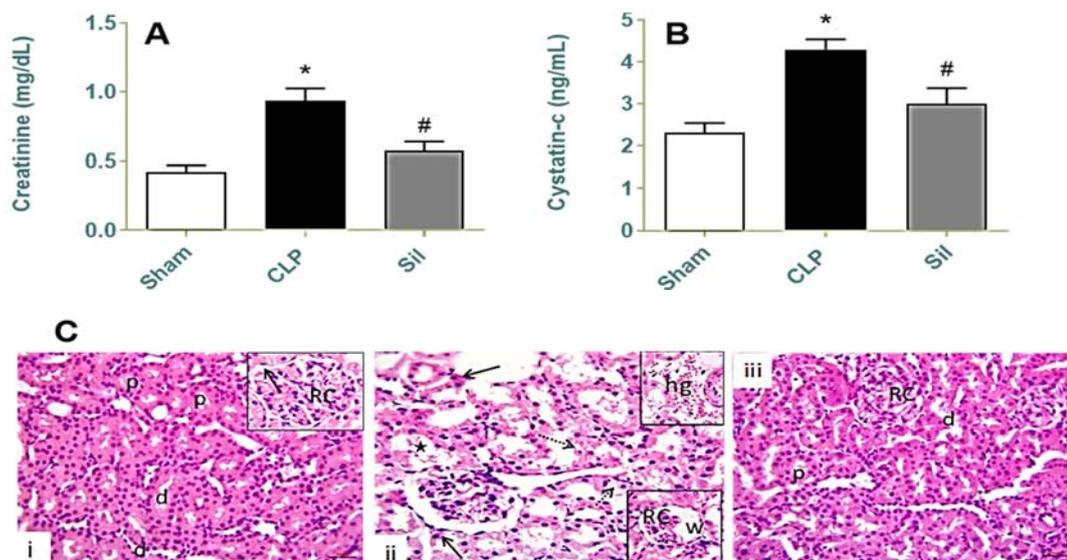
Figure 3: Effect of Silymarin treatment (100 mg/kg) on the kidney of septic rats. Effect of Silymarin on serum creatinine (**A**) and cystatin-c (**B**). Sil = Silymarin-treated, CLP = cecal ligation and puncture. Data were analyzed with one-way ANOVA followed by Tukey–Kramer test for multiple comparisons. *Significantly different from sham-operated rats ($p<0.05$); #Significantly different from untreated CLP group ($p<0.05$). C: Representative photomicrographs (H&E x400) of rat renal cortex from the sham control group (i) showing normal cortical architecture, normal structure of renal corpuscles (RC, inset), Bowman's space (arrow, inset), proximal (p) and distal (d) convoluted tubules (d). The untreated CLP group (ii) showing marked distortion and dilatation of renal tubules and epithelial lining either with apoptotic (arrows), vacuolated (dashed arrow), or desquamated with cellular debris in Lumina (stars). Notice the vascular congestion (C, left inset), interstitial hemorrhage (Hg, right lower inset), and shrunken distorted corpuscle (RC, inset) with widening of Bowman's space (W). (iii) Silymarin-treated CLP group showing restoration of normal structure of renal cortex with apparently normal corpuscles (RC), proximal (p) and distal (d) convoluted tubules (d).
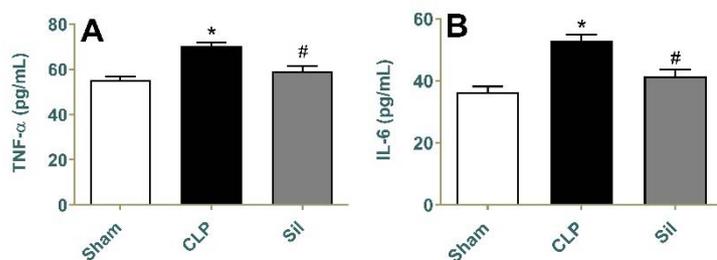
Figure 4: Effect of Silymarin treatment (100 mg/kg) on serum inflammatory markers of septic rats. Serum levels of the inflammatory cytokines TNF-α (A) and IL-6 (B) were determined by ELISA. Data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey–Kramer test for multiple comparisons. *Significantly different from sham-operated rats ($p<0.05$); #Significantly different from untreated CLP group ($p<0.05$).

3.5. Assessment of liver and kidney oxidative stress and antioxidant markers

Next, we evaluated the effect of silymarin on oxidative (MDA) and nitrative (NOx) stress parameters as well as on the endogenous antioxidant defense mechanisms (SOD and GSH) in liver and renal tissues. Induction of sepsis by CLP in rats increased hepatic and renal content of MDA and NOx in comparison with the sham-operated animals, indicative of increased sepsis-induced oxidative/nitrative stress. In line with these results, the activity of tissue SOD and the level of GSH were both lower than the results observed in the sham-operated animals ($p<0.05$). Contrarywise, treatment of the untreated CLP-operated rats with silymarin 1 h after surgery ameliorated these changes (Table 1) as it significantly reduced the levels of MDA and NOx when compared to the CLP group ($p < 0.05$). In addition, silymarin-treated rats showed conserved SOD and GSH levels.

Table 1: Effect of silymarin on liver and kidney oxidative and antioxidant profile of septic rats.

Parameter	Organ	Sham	CLP	Silymarin
NOx (nmol/mg protein)	Liver	13.2 ± 1.1	25.2 ± 1.5*	17.6 ± 1.7#
	Kidney	1.1 ± 0.1	5.0 ± 0.1*	2.2 ± 0.1*#
MDA (nmol/mg protein)	Liver	2.8 ± 0.2	9.9 ± 0.7*	3.7 ± 0.2#
	Kidney	2.1 ± 0.2	6.8 ± 0.5*	2.5 ± 0.2#
SOD (mU/mg protein)	Liver	1157 ± 43.2	227.6 ± 3.0*	713.4 ± 35.7*#
	Kidney	811.5 ± 14.6	189.8 ± 14.0*	758.0 ± 10.6*#
GSH (nmol/mg protein)	Liver	34.1± 3.0	15.2 ± 1.2*	31.7 ± 1.8#
	Kidney	10.2 ± 0.2	2.9 ± 0.2*	7.3 ± 0.5*#

Data were analyzed with one-way ANOVA followed by Tukey–Kramer test for multiple comparisons post-test, n = 6. *denotes significant difference compared to sham ($p<0.05$). #denotes significant difference compared to untreated CLP rats ($p<0.05$). CLP = cecal ligation and puncture, NOx = total nitrite/nitrate, MDA = malondialdehyde, SOD = superoxide dismutase, GSH = reduced glutathione.

4. Discussion

In this study, we tested the effect of silymarin against sepsis-induced mortality as well as its hepatorenal protective effects during sepsis. Based on its well-documented antioxidant and anti-inflammatory effects our original hypothesis was that silymarin would be protective against sepsis-induced organ damage and mortality. The results of the current study showed that silymarin in a dose of 100 mg/kg greatly improved the survival of septic rats. I effects have been attributed to scavenging of free radicals and increasing intracellular concentrations of reduced glutathione [25, 33].

The systemic inflammatory responses to infection is considered the most important cause of death during sepsis [2, 34]. Sepsis induces tissue damage not only by overproduction of reactive oxygen species, but also by triggering an aggravated local and systemic inflammatory responses leading to multiple organ failure [10, 35, 36]. Importantly, acute liver and kidney injuries following sepsis are associated with increased mortality morbidity in humans [19, 20, 37, 38]. The liver plays a critical role in preserving normal systemic homeostasis by detoxification and sterilization of bacteria, modulation of inflammation and regulation of blood coagulation [16]. Early in sepsis, liver dysfunction often occurs within 1.5 hours after CLP [39]. On the other hand, the kidney plays a critical role in the general circulation and organ perfusion through its direct contribution to sympathetic function, regulation of fluid and electrolytes and the renin angiotensin aldosterone system [40, 41]. Nevertheless, sepsis is the leading cause of acute kidney injury [42].

The cecal ligation and puncture (CLP) technique was followed in this study to induce polymicrobial sepsis in experimental rats. CLP in rodents is a well-established model of sepsis which is presently considered as the gold standard and the most widely utilized model in sepsis research [43-45]. The CLP technique is popular because it fits many of the important criteria of a good sepsis model including its simplicity, induction of polymicrobial sepsis, and the subsequent release of endotoxins [43, 46].

In this study, induction of sepsis by CLP resulted in increased mortality and deteriorated renal and hepatic function of septic rats. The hepatotoxic and reno-toxic effects of CLP were demonstrated as elevated serum liver enzymes (AST and ALT) as well as serum creatinine and cystatin-c, a marker of early

changes of kidney function and glomerular filtration rate [47]. Moreover, these results were augmented by histological evidence showing distorted renal and hepatic architecture and increased tissue damage in CLP rats. Our findings are in accordance with several previous reports demonstrating liver and kidney dysfunction in septic rats [12, 48, 49]. On the other hand, the decreased serum hepatic and renal damage markers, which was substantiated with beneficial histological findings in the silymarin-treated animals highlight its hepatorenal protective effects.

Oxidative stress has been implicated in the pathogenesis of sepsis-induced acute liver and kidney injury [4, 6, 7, 50, 51]. In the present study, induction of sepsis resulted in depletion of GSH, diminished SOD activity, and increased levels of MDA and NOx in both liver and kidneys of septic rats. Our results provide further evidence on the importance of increased oxidative/nitrative stress, at least in part, via impaired antioxidant defense mechanism in the pathogenesis of sepsis-induced multiple organ damage. Lipid peroxidation mediated by oxygen free radicals has been implicated in the pathogenesis of sepsis-induced tissue injuries [12, 52-54]. In line with its protective effects, rats given silymarin treatment had markedly lower MDA and NO levels and increased levels of GSH and SOD compared to the rats subjected to CLP without treatment in our study.

Several studies supported the antioxidant effect of silymarin; for example, it was reported that the protective effect of silymarin on a model of pancreatic damage induced by alloxan may be due to an increase in the activity of antioxidant enzymes SOD and GSH [55]. In another report [24], the authors observed a hepatoprotective effect of silymarin protection by enhancing hepatic GSH levels. Moreover, treatment by silymarin showed marked nephroprotection via mitigation of MDA and NO levels in a model of drug-induced nephrotoxicity [56]. In addition, the hepatoprotective effects of silymarin were attributed to its cell membrane stabilizing and lipid-preserving effects in a CCl4-induced liver toxicity model in rats [57]. Moreover, a more recent study suggested that the cytoprotective effects of silymarin can be attributed to normalization of lipid metabolism and preservation of mitochondrial energy production in high fat feeding-induced murine model of obesity [58]. Nevertheless, several studies illustrated the protective effects of other antioxidants such as N-acetylcysteine, resveratrol, vitamin E and curcumin on different organs following induction of sepsis [14, 49, 59, 60].

In the current study, treatment of septic animals with silymarin 1-h after induction reduced inflammation the important serum inflammatory cytokines TNF-α and IL-6, which were elevated in septic rats. Agents which inhibit cytokine release [61] or modulate cytokine action [62] have been found to limit organ damage induced by sepsis. Several inflammatory factors modulate the host response to severe sepsis. During or after experimental induction of sepsis, TNF-α and IL-6 are released from monocytes and endothelial cells; which causes free radical formation and oxidative tissue injury [62-64]. Silymarin protected human pancreatic β-cells against IL-1β- and IFN-γ-

induced NO production and β-cell dysfunction in a previous study [61]. These cytoprotective effects of silymarin were mediated through the suppression of c-Jun NH2-terminal kinase and Janus kinase/signal transducer and activator of transcription pathways (JAK/STAT) [61]. Furthermore, signalling by the proinflammatory cytokine IL-1β was inhibited by silymarin treatment in LPS-induced sepsis [65]. In another study, silymarin inhibited TNF-α-induced NF-kappa-B (NF-kB) activation, a mechanisms believed to be independent of its antioxidant activity [66]. Moreover, recent evidence suggested that that silymarin interferes with IL-6/STAT3 signaling [64], restores the Nrf2/HO-1 signaling axis [67], and reduces NF-κB expression [68]. Nonetheless, silibinin, a component of silymarin, inhibited NF-kB and NLRP3 inflammasome mediated signalling, which is responsible for the increased expression of inflammatory mediators such as interleukins, TNF-α, NO and prostaglandins [13, 64, 69].

5. Conclusion

In conclusion, the results of this study show that silymarin reduces sepsis-induced mortality in experimental animals, which was associated protection of liver and kidney against sepsis-induced acute organ damage. The hepatorenal protective effects of silymarin can be attributed, at least in part, to its ability to balance tissue oxidant-antioxidant status acting as a free-radical scavenger and lipid peroxidation inhibitor as well as induction of endogenous antioxidant mechanisms. In addition, silymarin-mediated protection of kidney and liver can also be attributed to its anti-inflammatory activity. Taken together, our results suggest a potential role of silymarin in the management of multiorgan failure during sepsis.

References

[1] Singer, M., et al., The third international consensus definitions for sepsis and septic shock (Sepsis-3). Jama, 2016. 315(8): p. 801-810.
[2] Font, M.D., B. Thyagarajan, and A.K. Khanna, Sepsis and Septic Shock – Basics of diagnosis, pathophysiology and clinical decision making. Medical Clinics of North America, 2020. 104(4): p. 573-585.
[3] Fleischmann, C., et al., Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. American journal of respiratory and critical care medicine, 2016. 193(3): p. 259-272.
[4] Galley, H.F., Oxidative stress and mitochondrial dysfunction in sepsis. British journal of anaesthesia, 2011. 107(1): p. 57-64.
[5] De Backer, D., et al., Pathophysiology of microcirculatory dysfunction and the pathogenesis of septic shock. Virulence, 2014. 5(1): p. 73-79.
[6] Khan, M.M., W.L. Yang, and P. Wang, ENDOPLASMIC RETICULUM STRESS IN SEPSIS. Shock, 2015. 44(4): p. 294-304.
[7] Vasco, C.F., et al., Sepsis-induced acute kidney injury: kidney protection effects by antioxidants. Rev Bras Enferm, 2018. 71(4): p. 1921-1927.
[8] Pavlakou, P., et al., Oxidative Stress and Acute Kidney Injury in Critical Illness: Pathophysiologic Mechanisms-Biomarkers-Interventions, and Future Perspectives. Oxid Med Cell Longev, 2017. 2017: p. 6193694.
[9] McGown, C.C., et al., ROCK induced inflammation of the microcirculation during endotoxemia mediated by nitric oxide synthase. Microvascular research, 2011. 81(3): p. 281-288.
[10] Singer, M., The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. Virulence, 2014. 5(1): p. 66-72.
[11] Levy, M.M., L.E. Evans, and A. Rhodes, The surviving sepsis campaign bundle: 2018 update. Intensive care medicine, 2018. 44(6): p. 925-928.

[12] Ahmed, A.-S.F., et al., Amelioration of Sepsis-Induced Liver and Lung Injury by a Superoxide Dismutase Mimetic; Role of TNF-α and Caspase-3. Journal of advanced Biomedical and Pharmaceutical Sciences, 2020. 3(1): p. 31-39.

[13] Tian, L., W. Li, and T. Wang, Therapeutic effects of silibinin on LPS-induced acute lung injury by inhibiting NLRP3 and NF-κB signaling pathways. Microbial pathogenesis, 2017. 108: p. 104-108.

[14] Atli, M., et al., The effects of selenium and vitamin E on lung tissue in rats with sepsis. Clinical and Investigative Medicine, 2012: p. E48-E54.

[15] Bagshaw, S.M., et al., Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. Intensive care medicine, 2009. 35(5): p. 871-881.

[16] Yan, J., S. Li, and S. Li, The role of the liver in sepsis. International reviews of immunology, 2014. 33(6): p. 498-510.

[17] Gustot, T., et al., Severe sepsis in cirrhosis. Hepatology, 2009. 50(6): p. 2022-2033.

[18] Boffa, J.-J. and W.J. Arendshorst, Maintenance of renal vascular reactivity contributes to acute renal failure during endotoxemic shock. Journal of the American Society of Nephrology, 2005. 16(1): p. 117-124.

[19] Hoste, E.A., et al., Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive care medicine, 2015. 41(8): p. 1411-1423.

[20] Alobaidi, R., et al. Sepsis-associated acute kidney injury. in Seminars in nephrology. 2015. Elsevier.

[21] Kaur, A.K., et al., Milk thistle (Silybum marianum): A review. IJPRD, 2011. 3: p. 1-10.

[22] Federico, A., M. Dallio, and C. Loguercio, Silymarin/Silybin and Chronic Liver Disease: A Marriage of Many Years. Molecules, 2017. 22(2).

[23] Rašković, A., et al., The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Molecules, 2011. 16(10): p. 8601-8613.

[24] Shaker, E., H. Mahmoud, and S. Mnaa, Silymarin, the antioxidant component and Silybum marianum extracts prevent liver damage. Food and Chemical Toxicology, 2010. 48(3): p. 803-806.

[25] Abdel-Moneim, A.M., et al., Free radical-scavenging, anti-inflammatory/anti-fibrotic and hepatoprotective actions of taurine and silymarin against CCl4 induced rat liver damage. PLoS One, 2015. 10(12).

[26] Demiroren, K., et al., A comparison of the effects of thymoquinone, silymarin and N-acetylcysteine in an experimental hepatotoxicity. Biomed Pharmacother, 2018. 106: p. 1705-1712.

[27] Medina, E., Murine Model of Polymicrobial Septic Peritonitis Using Cecal Ligation and Puncture (CLP), in Mouse Models for Drug Discovery: Methods and Protocols, G. Proetzel and M.V. Wiles, Editors. 2010, Humana Press: Totowa, NJ. p. 411-415.

[28] Bancroft, J.D. and M. Gamble, Theory and practice of histological techniques. 2008: Elsevier Health Sciences.

[29] Saller, R., et al., An updated systematic review with meta-analysis for the clinical evidence of silymarin. Forsch Komplementmed, 2008. 15(1): p. 9-20.

[30] Karimi, G., M. Ramezani, and Z. Tahoonian, Cisplatin nephrotoxicity and protection by milk thistle extract in rats. Evidence-Based Complementary and Alternative Medicine, 2005. 2(3): p. 383-386.

[31] Freitag, A.F., et al., Hepatoprotective effect of silymarin (Silybum marianum) on hepatotoxicity induced by acetaminophen in spontaneously hypertensive rats. Evidence-Based Complementary and Alternative Medicine, 2015. 2015.

[32] Shahbazi, F., et al., Potential renoprotective effects of silymarin against nephrotoxic drugs: a review of literature. Journal of Pharmacy & Pharmaceutical Sciences, 2012. 15(1): p. 112-123.

[33] Iqbal, B., et al., Silymarin-loaded nanostructured lipid carrier gel for the treatment of skin cancer. Nanomedicine (Lond), 2019. 14(9): p. 1077-1093.

[34] Vincent, J.-L., et al., Sepsis definitions: time for change. Lancet (London, England), 2013. 381(9868): p. 774.

[35] Pierrakos, C. and J.L. Vincent, Sepsis biomarkers: a review. Crit Care, 2010. 14(1): p. R15.

[36] Chung, K.K., Sepsis and multi-organ failure. Journal of Burn Care & Research, 2017. 38(3): p. 135-136.

[37] Asrani, S.K., et al., Burden of liver diseases in the world. Journal of hepatology, 2019. 70(1): p. 151-171.

[38] Montomoli, J., A. Donati, and C. Ince, Acute Kidney Injury and Fluid Resuscitation in Septic Patients: Are We Protecting the Kidney? Nephron, 2019. 143(3): p. 170-173.

[39] Recknagel, P., et al., Liver dysfunction and phosphatidylinositol-3-kinase signalling in early sepsis: experimental studies in rodent models of peritonitis. PLoS medicine, 2012. 9(11).

[40] Tokonami, N., et al., Local renal circadian clocks control fluid-electrolyte homeostasis and BP. J Am Soc Nephrol, 2014. 25(7): p. 1430-9.

[41] Eladari, D., et al., Electroneutral absorption of NaCl by the aldosterone-sensitive distal nephron: implication for normal electrolytes homeostasis and blood pressure regulation. Cell Mol Life Sci, 2014. 71(15): p. 2879-95.

[42] Peerapornratana, S., et al., Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. Kidney Int, 2019. 96(5): p. 1083-1099.

[43] Dejager, L., et al., Cecal ligation and puncture: the gold standard model for polymicrobial sepsis? Trends Microbiol, 2011. 19(4): p. 198-208.

[44] Baker, C.C., et al., Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. Surgery, 1983. 94(2): p. 331-335.

[45] Rittirsch, D., et al., Immunodesign of experimental sepsis by cecal ligation and puncture. Nature protocols, 2009. 4(1): p. 31.

[46] Toscano, M.G., D. Ganea, and A.M. Gamero, Cecal ligation puncture procedure. JoVE (Journal of Visualized Experiments), 2011(51): p. e2860.

[47] Feriozzi, S., et al., Cystatin C as a marker of early changes of renal function in Fabry nephropathy. Journal of nephrology, 2007. 20(4): p. 437-443.

[48] Liu, X., et al., Pterostilbene alleviates polymicrobial sepsis-induced liver injury: possible role of SIRT1 signaling. International immunopharmacology, 2017. 49: p. 50-59.

[49] Abu-Baih, R.H., et al., Natural polyphenols target the TGF-β/caspase-3 signaling pathway in CCl4-induced liver fibrosis in rats. Journal of advanced Biomedical and Pharmaceutical Sciences, 2019. 2(4): p. 129-134.

[50] Aydın, S., et al., Resveratrol protects sepsis-induced oxidative DNA damage in liver and kidney of rats. Balkan medical journal, 2016. 33(6): p. 594.

[51] Yu, Y., D. Tang, and R. Kang, Oxidative stress-mediated HMGB1 biology. Front Physiol, 2015. 6: p. 93.

[52] Şener, G., et al., Protective effect of β-glucan against oxidative organ injury in a rat model of sepsis. International immunopharmacology, 2005. 5(9): p. 1387-1396.

[53] Bedirli, A., et al., Beta-glucan attenuates inflammatory cytokine release and prevents acute lung injury in an experimental model of sepsis. Shock, 2007. 27(4): p. 397-401.

[54] Bar-Or, D., et al., Sepsis, oxidative stress, and hypoxia: Are there clues to better treatment? Redox Rep, 2015. 20(5): p. 193-7.

[55] Soto, C., et al., Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 2003. 136(3): p. 205-212.

[56] Guzel, S., et al., Potential renoprotective effects of silymarin against vancomycin-induced nephrotoxicity in rats. Drug Chem Toxicol, 2019: p. 1-7.

[57] Muriel, P. and M. Mourelle, Prevention by silymarin of membrane alterations in acute CCl4 liver damage. Journal of Applied Toxicology, 1990. 10(4): p. 275-279.

[58] Bin, F., et al., Silymarin protects against renal injury through normalization of lipid metabolism and mitochondrial biogenesis in high fat-fed mice. Free Radic Biol Med, 2017. 110: p. 240-249.

[59] Ritter, C., et al., Treatment with N-acetylcysteine plus deferoxamine protects rats against oxidative stress and improves survival in sepsis. Critical care medicine, 2004. 32(2): p. 342-349.

[60] Holthoff, J.H., et al., Resveratrol improves renal microcirculation, protects the tubular epithelium, and prolongs survival in a mouse model of sepsis-induced acute kidney injury. Kidney international, 2012. 81(4): p. 370-378.

[61] Matsuda, T., et al., Silymarin protects pancreatic β-cells against cytokine-mediated toxicity: implication of c-Jun NH2-terminal kinase and janus kinase/signal transducer and activator of transcription pathways. Endocrinology, 2005. 146(1): p. 175-185.

[62] Ibrahim, Y.F., et al., Tocilizumab attenuates acute lung and kidney injuries and improves survival in a rat model of sepsis via down-regulation of NF-κB/JNK: a possible role of P-glycoprotein. Inflammopharmacology, 2020. 28(1): p. 215-230.

[63] Coskun, A.K., et al., The effects of Montelukast on antioxidant enzymes and proinflammatory cytokines on the heart, liver, lungs, and kidneys in a rat model of cecal ligation and puncture–induced sepsis. The Scientific World Journal, 2011. 11: p. 1341-1356.

[64] Zheng, R., et al., Chemopreventive Effects of Silibinin on Colitis-Associated Tumorigenesis by Inhibiting IL-6/STAT3 Signaling Pathway. Mediators Inflamm, 2018. 2018: p. 1562010.

[65] Kang, J.S., et al., Protection against lipopolysaccharide-induced sepsis and inhibition of interleukin-1β and prostaglandin E2 synthesis by silymarin. Biochemical pharmacology, 2004. 67(1): p. 175-181.

[66] Chang, J.W., et al., Proinflammatory cytokine-induced NF-κB activation in human mesangial cells is mediated through intracellular calcium but not ROS: effects of silymarin. Nephron Experimental Nephrology, 2006. 103(4): p. e156-e165.

[67] Zhao, F., et al., Silymarin attenuates paraquat-induced lung injury via Nrf2-mediated pathway in vivo and in vitro. Clinical and Experimental Pharmacology and Physiology, 2015. 42(9): p. 988-998.

[68] Gupta, S.C., et al., Downregulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols. Archives of biochemistry and biophysics, 2014. 559: p. 91-99.

[69] Wu, J.-W., et al., Analysis of silibinin in rat plasma and bile for hepatobiliary excretion and oral bioavailability application. Journal of pharmaceutical and biomedical analysis, 2007. 45(4): p. 635-641.