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Protective and anti-inflammatory effects of silymarin on paraquat-induced nephrotoxicity in rats

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ABSTRACT

Introduction: Paraquat is a quaternary nitrogen herbicide which induces kidney toxicity due to producing oxidative stress. We have investigated the potential protective effects of silymarin on paraquat-induced renal toxicity.

Methods: Twenty-four male rats were divided into three groups, group 1, control group; group 2, rats that received paraquat only (25 mg/kg b.w./day, po); animals in group 3, was treated with paraquat (25 mg/kg b.w./day, po) and silymarin (50 mg/kg b.w./day, po). Then, the serum and tissue parameters of the oxidative stress and renal histopathological changes were examined.

Results: In group 2 which received paraquat only, a remarkable increase ($P<0.05$) was observed in serum creatinine, urea, malondialdehyde (MDA), protein carbonyl, and tumor necrosis factor alpha (TNF-α). Also, there was a significant decrease in renal superoxide dismutase, catalase (CAT), ferric reducing ability of plasma (FRAP) and vitamin C in the second group. Oral administration of silymarin significantly decreased serum urea, creatinine, protein carbonyl, MDA, and TNF-α as well as renal histopathological changes.

Conclusion: The present study suggests that silymarin has anti-inflammatory and nephroprotective effects against nephrotoxicity caused by paraquat.

Implication for health policy/practice/research/medical education:
Oral administration of silymarin could ameliorate renal histopathological changes in paraquat-induced nephrotoxicity. Also, it reduced serum urea, creatinine, protein carbonyl, and malondialdehyde. Silymarin has anti-inflammatory, nephro-protective and anti-oxidative stress activities. Hence, it might be useful in patients who were exposed to nephrotoxic agents.

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Introduction

Paraquat is a very toxic herbicide used widely across the globe. Paraquat exerts its herbicide effects through interfering with the electron transfer system and inhibiting the conversion of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. This herbicide causes toxicity through production of anion superoxide (O_2^-), which can led to the production of other reactive oxygen species (ROS), such as radical hydroxyl (OH^-) and hydrogen peroxide (H_2O_2) (1). Oxidative stress has key role in the toxicity of paraquat and leads to lipid peroxidation, body cell damage, and apoptosis (2). Paraquat is highly toxic for both humans and animals. It leads to some organ systems damage such as lung, liver, kidney, and Parkinson's disease (PD), as well (3-5). Paraquat-induced renal toxicity is highly common and reported as the first systemic effects of the paraquat toxicity. A rapid increase in serum creatinine level have been observed in previous reports of paraquat toxicity, which was indicating a decrease in kidneys glomerular filtration rate (GFR). Paraquat causes impaired renal function which is accompanied by reduction of the renal clearance rate and increasing of paraquat level, toxicity, and other organ dysfunctions (6,7). Administration of thioacetamide has been shown to induce liver and kidney injuries and silymarin ameliorated some parameters such as DNA fragmentation, nitric oxide, oxidative stress, collagen content, and liver and kidney histopathological changes in thioacetamide-induced toxicity (8). Silymarin is a potent antioxidant that can scavenge free radicals

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and ROS (9). Silymarin is a flavonolignan containing a combination of silibinin, silydianin and silychristin, which is extracted from the seeds of *Silybum marianum* (10,11). This antioxidant compound is known as a safe and healthy herbal product (12). Silymarin is used in treatment of liver disorders, such as cirrhosis, chronic liver inflammation, and renal disorders (13,14). In this study, we've investigated the protective effects of silymarin on serum urea, creatinine (Cr), malondialdehyde (MDA), renal MDA, catalase (CAT), superoxide dismutase (SOD), vitamin C, and TNF-α gene expression in paraquat-induced renal toxicity in rats.

Materials and Methods

Chemicals

Paraquat (200 g/L paraquat dicholoride) was purchased from Shandong Luba Chemical Co. Ltd., Jinan, China. Urea and Cr kits were purchased from Pars Azmoon Co. (Tehran, Iran). Sodium acetate and thiobarbituric acid were provided from Merck (Darmstadt, Germany). Riboflavin, nitro blue tetrazolium, vitamin C were obtained from Sigma-Aldrich Co. (St. Louis, Mo USA). All other chemicals used were analytical grade.

Animal treatment and experimental design

Twenty-four male Wistar rats (10-12 weeks old, 180-220 g), were divided into three groups (n=8/group). All animals were kept under normal laboratory conditions (22±2°C, 60±5% humidity, and 12:12 light dark cycle). Animals had access to standard rat pellet diet and water. The rats were divided randomly to three groups of 8 each, group 1 (Normal group) was orally given distilled water for 2 weeks. Group 2 (test group) was orally given paraquat only (25 mg/kg body weight/day) by gastric gavage for 2 weeks (15). Group 3 was orally administered paraquat (25 mg/kg body weight/day) and orally treated with silymarin (50 mg/kg body weight/day, po) (16) at an interval of 1 hour for 2 weeks.

After 2 weeks, rats were anesthetized with chloroform and blood specimens collected by cardiac puncture method to separate serum and plasma. Also, kidney sample was removed to determine tumor necrosis factor-α (*TNF-α*) gene expression, kidney CAT, SOD, and histopathological examinations.

Biochemical analysis

Urea and Cr were measured by enzymatic method with auto analyzer system (BT3000, Rome, Italy). Serum TNF-α was evaluated by enzyme-linked immune sorbent assay (ELISA) kit, (Bioassay technology laboratory Shanghai, China).

Serum and renal MDA levels were determined as described previously (17). Plasma antioxidant capacity was estimated by ferric reducing ability of plasma (FRAP) method as described previously (17).

Renal CAT activity was measured by previously described method (18). The activity of the renal SOD was determined in the renal tissue by Beauchamp and Fridovich method (19) and total protein was determined by Bradford method (20).

Renal vitamin C level in the experimental groups was estimated by Stanley and Omaye method (21) and *TNF-α* gene expression was evaluated by real-time quantitative PCR (RT-qPCR) and the ΔΔCT method as described previously (22). β-actin was used as internal control for mRNA expression and normalizing data. The serum protein carbonyl level was assessed by Reznick and Parker's spectrophotometric method (23).

Kidney histopathological studies

Kidney sample in each rat fixed with formalin 20% for histopathological examination. After paraffin embedding, sections prepared at 5 μm thickness, stained by hematoxylin-eosin (H&E) (24), and histological changes were examined by optical microscope.

Statistical analysis

The results were expressed as mean ± SD. Analysis of the results was performed using one-way ANOVA by SPSS 20.0 (SPSS Inc., Chicago, IL, USA), and Tukey's post hoc test was used for multiple comparisons. Values of $P<0.05$ were considered significant.

Results

Effects of silymarin on serum urea, Cr, MDA, plasma FRAP and renal MDA levels

Table 1 shows the effects of paraquat and silymarin on serum urea, Cr, MDA and renal MDA levels in the experimental groups. Administration of paraquat in the second group (group receiving only paraquat) led to a significant elevation ($P<0.05$) in serum urea, Cr, and MDA and renal MDA levels in comparison with the control group (Table 1). There was a remarkable reduction ($P<0.05$) in serum urea, Cr, and MDA and renal MDA levels of the group treated with silymarin comparing with the second group ($P<0.05$).

Group 2 has shown a significant reduction ($P<0.05$) in FRAP level after receiving paraquat in comparison with the control group (Table 1). There was a significant increases ($P<0.05$) in the plasma FRAP level of group which treated by silymarin when compared with the second group (group receiving only paraquat).

Effects of silymarin on renal CAT and SOD activities

Figure 1 shows that paraquat significantly decreased ($P<0.05$) renal CAT and SOD activities compared with the control group. Oral administration of silymarin led to an increase ($P<0.05$) in renal CAT and SOD activities compared to the second group (group receiving only paraquat).

Table 1. Effects of silymarin on serum urea, Cr, MDA, plasma FRAP and kidney MDA in paraquat-induced renal injury

Parameters	Group 1	Group 2	Group 3
Cr (mg/dL)	0.42 ± 0.04	0.66 ± 0.05[a]	0.43 ± 0.05[b]
Urea (mg/dL)	45.37 ± 3.81	67.50 ± 2.07[a]	55.50 ± 3.33[a,b]
FRAP (µM)	518.38±36.14	393.13±29.06[a]	529.88±41.22[b]
Serum MDA (µM)	9.99±1.05	19.95±1.21[a]	11.13±0.94[b]
Kidney MDA (µmol/mg protein)	1.88±0.03	4.87±0.03[a]	2.50±0.03[a,b]

Abbreviatios: FRAP, ferric reducing ability of plasma; MDA, malondialdehyde; Cr, creatinine.
Values are expressed as mean ± SD. Group 1, normal control; group 2, receiving paraquat only; group 3, rats receiving paraquat and silymarin.
[a]$P < 0.05$ compared to group 1.
[b]$P < 0.05$ compared to group 2.

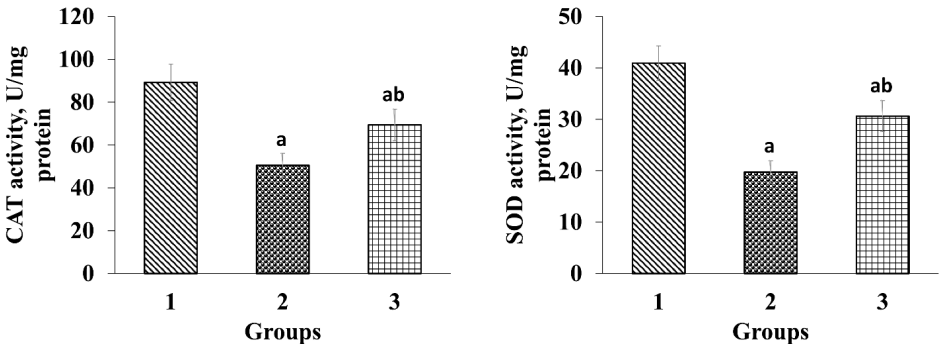

Figure 1. Effects of silymarin on CAT and SOD activities. Values are expressed as mean ± SD and n=8 in each group. Group 1, normal control; group 2, received paraquat only; group 3, rats supplemented with paraquat and silymarin.
[a]$P < 0.05$ compared to group 1.
[b]$P < 0.05$ compared to group 2.

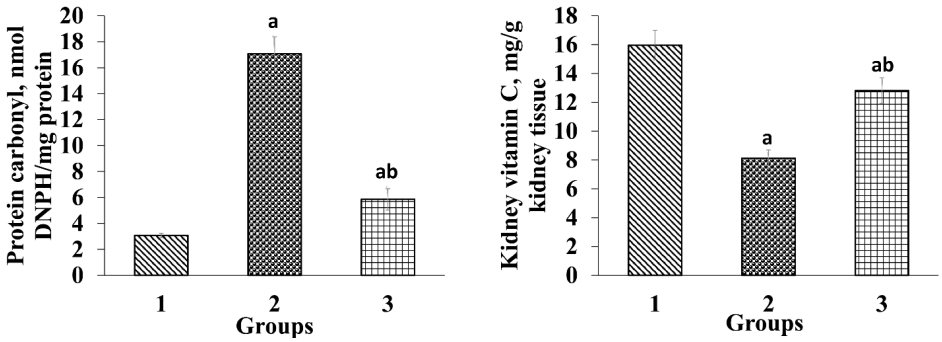

Figure 2. Effects of silymarin on serum PC and vitamin C levels. Values are expressed as mean ± SD and n=8 in each group. Group 1, normal control; group 2, received paraquat only; group 3, rats supplemented with paraquat and silymarin.
[a]$P < 0.05$ compared to group 1.
[b]$P < 0.05$ compared to group 2.

Effects of silymarin on the renal vitamin C and PC levels

Figure 2 shows the effects of silymarin on the renal vitamin C and PC levels. Oral administration of paraquat in the second group led to a significant decrease ($P < 0.05$) in the renal vitamin C level compared to the control group. On the other hand, administration of silymarin has increased the renal vitamin C level significantly compared to the paraquat group ($P < 0.05$). Also, there was a significant increase ($P < 0.05$) in serum PC levels in rats receiving only paraquat when compared to the control group (Figure 2). Nevertheless, administration of silymarin has significantly reduced ($P < 0.05$) the serum PC level in comparison with the second group.

Effects of silymarin on serum and renal *TNF-α* gene expression

Figure 3 shows the effects of silymarin on serum and renal TNF-α levels. In the second group (group receiving only

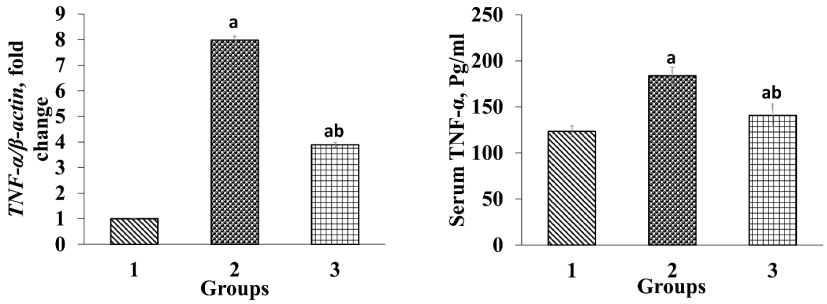

Figure 3. Effects of silymarin on serum TNF-α and expression of *TNF-α* gene. Values are expressed as mean ± SD and n=8 in each group. Group 1, normal control; group 2, received paraquat only; group 3, rats supplemented with paraquat and silymarin.
[a]$P < 0.05$ compared to group 1.
[b]$P < 0.05$ compared to group 2.

paraquat), administration of paraquat led to a significant increase ($P<0.05$) in serum TNF-α levels and its renal gene expression than the control group. Oral administration of silymarin could significantly decrease ($P<0.05$) serum and renal *TNF-α* gene expression compared to the second group (group receiving only paraquat) (Figure 3).

Histopathological findings

Microscopic studies of the second group (group receiving only paraquat) have shown lymphocyte infiltration compared to the control group (Figure 4). In the group treated with silymarin, a significant decrease in lymphocyte infiltration was seen compared to the second group (group receiving only paraquat).

Discussion

Paraquat is secreted by proximal tubules before renal damage, but tubular damages happen with an increase in paraquat concentration. In paraquat-induced renal toxicity, primarily the glomerular coatings (podocytes) are damaged; then, tubular degeneration, and the formation of eosinophilic granular cytoplasm is happen in distal and proximal tubules (25-27).

Paraquat-induced nephrotoxicity is a common causes of mortality in this group of patients (28). Urea and Cr are among the markers of renal toxicity workup (29). In this study, there is an increase in serum urea and Cr levels in group 2 (Table 1), which indicates the paraquat-induced renal damage; which was consistent with previous studies (27,29,30). In our study, administration of silymarin led to protect kidneys against paraquat toxicity and reduces serum urea and Cr levels (Table 1). According to previous reports, silymarin can reduce urea and Cr (10,13,31). Therefore, these effects of silymarin on paraquat-induced nephrotoxicity may be produced, at least in part, due to its antioxidant properties.

Paraquat toxicity caused by production of free radicals and oxidative agents (32). There are antioxidant systems against oxidative agents in human body. CAT and SOD are considered as enzymatic antioxidants (33). SOD converts superoxide anion into hydrogen peroxide; and CAT converts hydrogen peroxide into oxygen and water (34). In current study, the SOD and CAT levels were decreased (Figure 1), which is in accordance with previous studies that paraquat shown the ability to decrease the SOD and CAT level in kidney tissue (4). Nevertheless, in the present study silymarin administration led to improve the activity of SOD and CAT (Figure 1) and increase their antioxidant effects which is consistent with a previous study (14). Therefore, these effects of silymarin on renal SOD and CAT in paraquat-induced nephrotoxicity may be due to its antioxidant properties.

Vitamin C is a non-enzymatic antioxidant and can protect body against the oxidant agents. There are reports of low vitamin C levels in paraquat-induced renal toxicity in previous studies (35). Our findings shown a reduction of vitamin C levels in paraquat-induced renal toxicity, as well (Figure 2). Nevertheless, silymarin could reduce the toxic effects of paraquat and consequently increases vitamin C levels due to its antioxidant properties. On

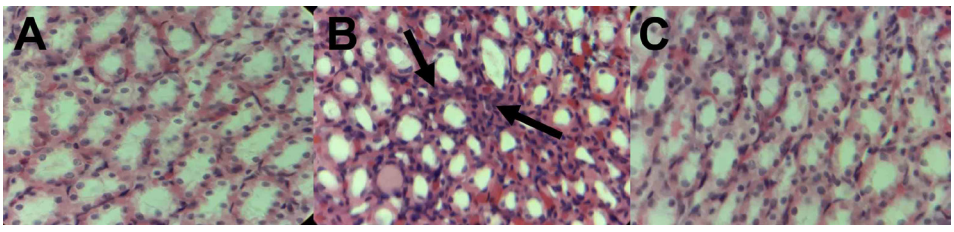

Figure 4. Effects of silymarin on histopathological changes in kidney tissues in rats. **A**, the normal group (group 1). **B**, rats treated with paraquat only (group2) showing pathological changes in the kidney such as mononuclear cell infiltration. The black arrows show lymphocyte infiltration. **C**, (group 3) paraquat-administered rats supplemented with silymarin (50 mg/kg body weight).

the other hand, paraquat can induce lipid oxidation and protein carbonyl (PC) production through ROS (36,37). MDA is considered as a lipid peroxidation biomarker (38). In the present study, paraquat administration led to increase both the serum and renal MDA and serum protein carbonyl levels by producing oxidant agents which is consist with previous studies (25,29,35). Silymarin administration, however, has reduced serum PC and serum and renal MDA levels (Figure 2), which could be an indication of silymarin effects on inhibiting the oxidation of proteins and lipids, which is consistent with previous results (9,39). Also, our results showed that silymarin have increased the FRAP level (Table 1), which may be due to the silymarin high levels of antioxidant activity, and could justify an increase in SOD and CAT activities and a decrease in MDA and PC by silymarin. In previous studies, an increase in FRAP has been observed by administration of silymarin, as well (40).

Oxidative stress can activate the main mediator of immunity nuclear factor-kappa β (NF-κβ), which is expressed in excessive inflammation and increases pro-inflammatory cytokines such as TNF-a, IL-1b, IL-6, and COX-2. It is reported that production of TNF-α in the kidney is responsible for inflammation of the kidney through inducing apoptosis pathway and tubular necrosis (3,41,42). TNF-α is an inflammatory marker and known macrophagic cytokine (43,44). Also, previous studies have shown that paraquat-induced renal toxicity is caused by oxidative stress and inflammation, and ROS plays a role in this toxicity (25). Our findings shown that paraquat increased the serum and renal *TNF-α* gene expression; which is consistent with previous study (3). Nevertheless, in our study there was a significant decrease in serum and *TNF-α* gene expression in the silymarin treatment group (Figure 3). Previous studies have also shown that silymarin reduces TNFα in the presence of renal damage (14). Our histopathologic tests have shown that silymarin reduces infiltration of lymphocytes, apoptosis, and inflammation (Figure 4). Silymarin protects the kidneys against damage and toxicity through reducing the expression of inflammatory factors such as TNF-α.

Conclusion

Silymarin exhibits protective effect against paraquat – induced kidney injury. Silymarin increases the level of enzymatic (SOD and CAT) and non-enzymatic (Vit C) antioxidants in the kidney and reduces the expression of inflammatory factors such as TNF-α. Silymarin is considered an appropriate antioxidant against oxidative stress.

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Authors' contributions

EH and ASR contributed in conception, design, data collection, statistical analysis and preparation of the manuscript. EH confirmed the final version of the manuscript for publication.

Conflict of interests

The authors declare that there is no conflict of interest.

Ethical considerations

Ethical issues have been observed by the authors. All procedures were approved by the Ethics Committee of Shahrekord University of Medical Sciences, Shahrekord, Iran (Ethic number IR. SKUMS. REC. 1395. 151).

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