Kerbala Journal of Veterinary Medical Sciences Issue (3), Volume (2), (2026)

Protective Effects of Milk Thistle Extract on Physiological Functions, and Oxidative Stress in Chromium(VI)-Induced Liver and Kidney Injury

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Received:14/8/2026 **Accepted:** 5/9/2026 **Published:** 6/9/2026

***Abstract*—** Industrial effluents/ wastewater is a primary source of hexavalent chromium (Cr(VI)) contamination in the environment. This pollutant, widely used in various industries, is highly toxic and persistent, making it one of the most significant global environmental concerns. Its hexavalent form is carcinogenic, genotoxic, and mutagenic, with exposure linked to respiratory issues, organ damage, and DNA mutations. However, milk thistle, a plant with traditional medicinal use, can eliminate harmful free radicals and regulate inflammatory reactions. Milk Thistle has showed protective and treated effects in different models of disease. Thus, we hypothesized that silymarin would be protective against chromium(VI) induced liver and kidney injury.

Twenty four male albino rats were divided into four groups and allocated as control group (G1)was treated orally with normal saline for 4 weeks, milk thistle aqueous extract treated group (G2) received potassium dichromate to induce toxicity (2 mg/kg/ day for 4 weeks), G3 received a daily intake of 200 mg/kg milk thistle for 4 weeks, and G4 received both potassium dichromate and milk thistle extract. Biochemical analysis for blood samples as liver function (ALT, AST and ALP), kidney function (urea and creatinine) and lipid peroxidation (MDA) and antioxidant Capacity (CAT). The exposure to Cr(VI) increased malondialdehyde (MDA), ALT, AST, ALP, urea, creatinine, while reducing antioxidant markers levels, indicating oxidative stress, liver dysfunction. Protective role of milk thistle extract with Cr(VI) (G4) resulted in significant improvements, marked by significant increase in the levels of antioxidant enzymes, decreased levels of MDA, ALT, AST, ALP, urea, creatinine, in the serum. Milk thistle extract relieve Cr(VI)-induced hepatic and renal toxicity, via increasing antioxidants and decreasing lipid peroxidation

Keywords **—** Milk thistle , Hexavalent chromium, Antioxidants, Potassium dichromate, Lipid Peroxidation.

INTRODUCTION

Hexavalent chromium Cr(VI) poses significant health risks due to its oxidative properties and its ability to induce cellular damage. As it is largely non-biodegradable, Cr(VI) tends to persist in the environment, leading to long-term contamination of soil and water, as well as serious health hazards for both humans and wildlife. Within living organisms, the hexavalent form of chromium is recognized as carcinogenic, genotoxic, and mutagenic(1,2).

Potassium dichromate ($K_2Cr_2O_7$) is a representative example of a hexavalent chromium compound. This highly hazardous molecule is widely used in several industrial processes (3). It exerts multiple detrimental health effects as an oxidizing agent. Upon exposure, it is reduced by various cellular metabolites, generating trivalent chromium intermediates. This reduction process triggers oxidative stress, resulting in damage to cellular lipids, proteins, and DNA due to the production of reactive species (4). Exposure to potassium dichromate has been associated with numerous adverse health outcomes, including hepatotoxicity, nephrotoxicity, carcinogenicity, as well as cardiovascular and neurological disorders (5).

Traditional medicine has long utilized the seeds of Silybum marianum, commonly known as milk thistle. This annual or biennial plant, belonging to the Asteraceae family (6), has been employed for over 2,000 years to treat various liver, kidney, and gallbladder ailments, such as hepatitis, cirrhosis, and jaundice. Milk thistle extract contains silymarin, a compound with well-documented antioxidant and anti-inflammatory effects. It has been shown to reduce oxidative stress, lipid peroxidation, insulin resistance, mitochondrial dysfunction, and cellular necrosis induced by chemical intoxication. Recent research has focused on its potential to protect the liver against toxic chemicals and environmental pollutants. The cytoprotective benefits of silymarin are primarily attributed to its antioxidant and free-radical-scavenging capabilities (7).

In the present study, the efficacy of milk thistle extract in mitigating the detrimental effects of Cr(VI) on the liver and kidneys of male albino rats was investigated. Although milk thistle tea is commonly used as an herbal remedy for liver ailments, the specific protective role of its extract against potassium dichromate induced oxidative stress and tissue damage in the liver and kidneys of male albino rats has not been fully established. Therefore, this study aimed to

determine whether milk thistle extract could reduce such oxidative damage.

MATERIALS AND MTHODS

Chemicals and extract preparation

This study's methodology and materials included potassium dichromate supplied from an Indian company and milk thistle extract was obtained by infusing 100 g of freshly ground milk thistle seeds obtained from a Karbala local market with 1200 ml of boiling water at 100 C for 10 minutes (8). The extract was then sieved and heated to 40 °C before being dried and stored at -20°C. The extract was administered at dosages equivalent to the therapeutic dose.

Animals and experimental design

This study employed twenty-four adult male albino rats at the College of Veterinary Medicine, University of Kerbala, Iraq. Their average weight ranged from 200 to 250 grams, and their ages varied from 14 to 16 weeks.

The rats were left for two weeks to acclimatize in groups of four each cage in an animal home before the experiment beginning of the experiment, with unlimited water and food available to them. The animal housing provided an environment at 20-25°C and a natural lighting cycle.

The rats were separated into four groups (6 rats/ group):

Group (G1)- Control: the animals were given regular normal saline for 4 weeks orally.

Group (G2)- Potassium Dichromate: rats were received 2 mg/kg of body weight of potassium dichromate intraperitoneally once a day for 4 weeks.

Group (G3)- Milk Thistle extract: rats were given 200 mg/kg/day of milk thistle extract orally for 4 weeks.

Group (G4)- potassium dichromate and milk thistle extract: rats were given 2 mg of potassium dichromate /kg/day intraperitoneally for 4 weeks, with 200 mg/kg/day of milk thistle extract orally for 4 weeks.

At the end of the experiment, the rats were euthanized by placing them in a sealed container saturated with carbon dioxide (CO_2) gas. Blood samples (5 mL) were collected from rats that had been fasted overnight, using cardiac puncture, and then the samples were centrifuged at 3000 revolutions per minute (RPM) for 15 minutes to separate the serum. The clear serum samples were stored in Eppendorf tubes at −20 °C for subsequent analysis.

Liver functions

The levels of alanine aminotransferase enzyme (ALT), aspartate aminotransferase enzyme (AST), and alkaline phosphatase enzyme (ALP) using enzymatic method(9).

Kidney functions

A commercial kit was used to measure the amount of urea in accordance with Fawcett and Scott (10) instructions, while a different kit from the same supplier was used to determine the quantity of creatinine in accordance with Henry(11) kinetic colorimetric method.

Oxidative stress levels

respectively Assessing Oxidative Stress: Lipid Peroxidation and Antioxidant Capacity in the blood catalase enzyme (CAT), and polyunsaturated fatty acids peroxidation marker malondialdehyde (MDA) activity were measured using test kits.

STATISTICAL ANALYSIS

A statistical software program (SPSS) version 20.0 for windows (SPSS Inc., Chicago, IL, USA) with a significant difference was used to perform a one-way ANOVA followed by a LSD multiple comparison test to determine differences between groups. The mean value along with the standard deviation was used to express the data.

RESULT AND DISCUSSION

As presented in Table 1, milk thistle extract ameliorated potassium dichromate induced liver dysfunction. Serum levels of ALT, AST, and ALP in the milk thistle only group (G3) did not differ significantly from those in the control group (G1). In contrast, rats treated with potassium dichromate alone (G2) showed markedly elevated liver enzyme levels compared to G1 ($p < 0.05$). Co-administration of milk thistle extract with potassium dichromate (G4) significantly reduced enzyme levels relative to G2 ($p < 0.05$); however, levels in G4 remained significantly higher than those in G1.

Table 1. Comparison in the serum biomarkers level (liver function) in rat after exposure to dichromate (G2), milk thistle extract (G3) dichromate and milk thistle extract (Group 4) with the control group (G1) .

Groups		parameters (Mean ± SD)		
		AST (U/L)	ALT (U/L)	ALP (U/L)
G1	Control	84.7± 6.84[b]	45.4± 4. 14 [b]	152.4±9.90[b]
G2	Potassium dichromate	142.6±5.52[a]	86.1±6. 17[a]	203.5±9.72[a]
G3	Milk thistle	76.7 ± 4.64[b]	42.9±3.15[b]	145.7±8.73[b]
G4	Potassium dichromate + milk thistle	96.3 ± 8.47[c]	61.7± 3.23[c]	170.8 ± 7.5[c]
LSD		5.99	3.98	8.26

As presented in Table 2, milk thistle extract partially protected against potassium-dichromate-induced kidney damage. Milk thistle alone (G3) did not significantly alter urea levels but caused a mild, significant increase in creatinine compared to controls ($p < 0.05$). Potassium dichromate alone (G2) significantly elevated both urea and creatinine ($p < 0.05$). Co-treatment with milk thistle extract (G4) significantly reduced both markers compared to G2, though values remained above control levels.

Table 2. Comparison in the serum biomarkers level (kidneys function) in rat after exposure to dichromate (G2), milk thistle extract (G3) dichromate and milk thistle extract (G4) with the control group (G1) .

Groups		parameters (Mean ± SD)	
		Urea (mg/dl)	Creatinine (mg/dl)
G1	Control	39.1± 3.74^{c}	0.41 ± 0.04^{d}
G2	Potassium dichromate	61.6 ± 2.72^{a}	1.12 ± 0.07^{a}
G3	Milk thistle	35.7 ± 1.85^{d}	0.59 ± 0.05^{c}
G4	Potassium dichromate + milk thistle	44.3 ± 2.67^{b}	0.71 ± 0.03^{b}
LSD		2.58	0.045

As shown in Table 3, milk thistle extract alleviated oxidative stress induced by potassium dichromate. Dichromate alone (G2) significantly increased MDA levels and decreased CAT activity compared to controls ($p < 0.05$). Milk thistle alone (G3) significantly decreased MDA and increased CAT activity relative to controls ($p < 0.05$). Co-treatment with milk thistle extract (G4) significantly reduced MDA and restored CAT activity compared to G2 ($p < 0.05$), though levels did not fully return to control values.

Table 3. Comparison in the serum biomarkers level in rats after exposure to dichromate (G2), milk thistle extract (G3) dichromate and milk thistle extract (G4) with the control group (G1) .

Groups		parameters (Mean ± SD)	
		MDA (nmol/ml)	CAT (U/L)
G1	Control	29.1± 4.84^{c}	30 ± 5.14^{b}
G2	Potassium dichromate	51.6 ± 3.52^{a}	18.12 ± 5.17^{d}
G3	Milk thistle	25.7 ± 2.64^{d}	35.59 ± 6.15^{a}
G4	Potassium dichromate + milk thistle	34.3 ± 4.47^{b}	25.71 ± 6.23^{c}
LSD		3.63	5.23

The toxicological profile of chromium(VI) underscores its potential for causing serious health effects through various biological mechanisms. Cr (VI) pollution has become one of the world's most serious environmental concerns due to its long persistence in the environment and highly deadly nature in living organisms. (12) The liver and the kidney are among the most vulnerable and early affected organs during chromium(VI) exposure. Cr(VI)-induced liver dysfunction is considered a contributing factor to multiple organ dysfunction and Cr(VI)-induced death.(13) The mechanisms of Cr(VI) toxicity are multifaceted, involving oxidative stress, inflammation, and genotoxic effects. (14) In addition to, it causes toxicity in a variety of ways, It can reduce immune system activity or efficiency, compete with enzyme activity cofactor fixation sites, suppress important enzymes such as oxidative phosphorylation, and cause changes in cell architecture, notably in the lipoprotein region of the membrane(15).

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 (16). Like other flavonoids, silymarin exhibits various pharmacological and therapeutic applications due to its antioxidant, free radical scavenger and anti-inflammatory properties that could potentially mitigate the harmful effects of chemical toxins (17). Thus, based on the potent antioxidant and anti-inflammatory effects of silymarin, we hypothesized that silymarin would improve the survival rats and exert a protective effect against Cr(VI)-induced liver and kidney injury.

Previous studies have indicated that the exposure of potassium dichromate leads to inflammation and oxidative damage due to the generation of free radicals which promotes oxidative degradation of lipids and reduces the body's antioxidant defense mechanism(18). This study highlights the nephrotoxic effects of potassium dichromate (G2) resulted in a significant elevation in malondialdehyde (MDA) levels, which is a marker of lipid peroxidation. This elevation was paralleled with a significant decline in the levels of catalase enzyme (CAT), indicating a compromised antioxidant defense mechanism in scavenging free radicals. As a result, the liver and kidney were subjected to pathological damage caused by these free radicals. These results agree with those stated by El Menyiy, Al Waili (19), who demonstrated that exposure to potassium dichromate leads to oxidative damage as verified by rise in MDA levels and decline in CAT levels. The decrease in levels of CAT can cause an accumulation of free radicals, resulting in lipid peroxidation, increased cell membrane permeability, complete cell disintegration, and the release of cellular components into the bloodstream(20). The current study revealed that intoxication with potassium dichromate (G2) caused oxidative damage to hepatocytes, resulting in elevated blood levels of ALT, AST, ALP. These results are in line with earlier researches that reported similar effects of potassium dichromate on hepatocytes by increased levels of hepatic enzymes in the bloodstream(21). Additionally, potassium dichromate toxicity induced bile duct hyperplasia and ALP accumulation in the blood (22). The current study found that the liver of rats exposed to potassium dichromate (G2) exhibited blood vessel congestion, which matching with the results of previous research after treatment experimental animals with potassium dichromate and caused histopathological changes in liver tissue(23).

The damaging effects of potassium dichromate on the kidneys, which frequently lead to renal failure and acidosis, have been documented in several investigations. Furthermore, elevated MDA levels further promote the generation of reactive oxygen species (ROS) in renal cells, leading to necrosis and the release of kidney enzymes into the bloodstream (24). In the current research, potassium dichromate intoxication in rats (G2) caused several forms of renal damage, as well as elevated levels of urea, creatinine, in the serum. These findings are consistent with previous researches, that reported similar outcomes of renal corpuscle

widening, glomerular degeneration, and cellular infiltration in rats treated with potassium dichromate(25). In view of that, urea and creatinine can accumulate in the bloodstream as a result of potassium dichromate -induced renal failure, along with accompanying problems like acidosis and changes in electrolytes. In the present study potassium dichromate toxicity (G2) caused renal damage through multiple mechanisms, including changes in the concentrations of antioxidant enzymes, and inflammatory reactions. Previous studies have shown that potassium dichromate intoxication causes elevated serum levels of calcium and phosphorus, which can lead to failure in the renal tubules by formation of calcium phosphate and calcium oxalate crystals, resulting in widening of the proximal convoluted tubules, tubular epithelium necrosis, edema, and interstitial inflammation(26). In the current study, rats treated with both Potassium dichromate and milk thistle extract (G4) had restored redox balance, as evidenced by lower MDA and higher CAT levels when compared to potassium dichromate -only treated rat. These results suggest that milk thistle extract's antioxidant and anti-inflammatory properties may have played a role in reversing potassium dichromate -induced oxidative damage(27). In the present study, milk thistle extract improved liver and kidney function in potassium dichromate treated rats (G4), as well as recorded decrease in levels of ALT, AST, ALP in the serum. This confirms its ability to reduce lipid peroxidation, minimize cell necrosis and enzyme leakage. Previous studies also showed milk thistle extract's hepatorenal protective effects against lead acetate, carbon tetrachloride, and sepsis-induced oxidative damage through normalization of liver and kidney enzymes and proteins serum levels(28). Earlier research has demonstrated that milk thistle extract (silymarin) can protect against oxidative damage in animal models treated with paraquat (29) and busulfan by decreasing lipid peroxidation (MDA), enhancing antioxidant defense mechanisms (CAT), and improving liver and kidney parameters in potassium dichromate -treated rats (G4). Its antioxidant enzymes and anti-inflammatory reactions may have prevented kidney dysfunction, consistent with previous studies(30). Researchers found that milk thistle extract protected cells from potassium dichromate-induced oxidative stress. Milk thistle had a protective effect to liver and kidney for the reduction of MDA levels and the enhancement of catalase activity and superoxide dismutase(31).

CONCLUSION

Milk thistle extract, particularly silymarin, has been shown to mitigate the toxic effects of potassium dichromate on the liver and kidneys by boosting antioxidant levels and reducing lipid peroxidation. In experimental rats, silymarin effectively countered the harmful impacts of potassium dichromate on liver and kidney function, as well as serum markers. The antioxidant properties of silymarin helped restore cellular balance and minimize tissue damage. These findings suggest that milk thistle extract could serve as a therapeutic and preventive strategy for potassium dichromate poisoning. However, additional studies are needed to determine if these benefits extend to human health, highlighting the potential of milk thistle as a protective agent against toxic substances.

Acknowledgements

N/A

Conflict of Interest

The authors declare no conflict of interest.

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