Effects of Silymarin on Cadmium-Induced Toxicity in Rats

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Abstract

Background: Cadmium has toxicological significance and there is no effective therapy for its poisoning.

Objective: The effects of silymarin on the parameters indicative of cadmium-induced toxicity were studied in rats.

Methods: 130 adult male Wistar rats were divided into 13 groups each comprising 10 rats. 1 group as control group was not administered neither cadmium nor silymarin. Cadmium chloride (3mg/kg/week) was administered intraperitoneally to 12 groups for 6 weeks. The 12 groups were divided into two sets of 6 groups. In the first set, one group was kept as control and silymarin in the doses of 15, 30, 60, 120 and 240 mg/kg/week was administered orally to each group for 6 weeks. In the second set, one group was kept as control and the aforementioned doses of silymarin were administered orally to each group for 6 weeks after 6 weeks of cadmium administration.
Blood samples were taken after 6 weeks from the first set and after 12 weeks from the second set to determine AST (aspartate aminotransferase), ALT (alanine aminotransferase) and ALP (alkaline phosphatase) levels and catalase activity.

Results: In the first set in all silymarin treated groups, ALP level significantly decreased compared with control and in the second set, AST level decreased significantly compared with control only in groups treated with high doses of silymarin. Different doses of silymarin except the dose of 15 mg/kg significantly increased serum catalase activity compared with control in both sets.

Conclusion: Silymarin prevents and reverses cadmium-induced toxicity possibly through its anti-oxidative property in rats.

Keywords: ***Silybum marianum*, Silymarin, Cadmium toxicity, Rat**

Introduction

Cadmium is extremely toxic and a carcinogen. Cadmium is ranked close to lead and mercury as a metal of current toxicological concern. Cadmium is used in electroplating and galvanization and in plastics, paint pigments (cadmium yellow) and nickel-cadmium batteries. Cadmium is also present in cigarette smoke. Because <5% of the metal is recycled, environmental pollution is an important consideration. Coal and other fossil fuels contain cadmium and their combustion releases the element into the environment. Extraction and processing of zinc and lead also lead to environmental contamination with cadmium. Workers in smelters and other metal-processing plants may be exposed to high concentrations of cadmium in the air; however, for most of the population, food is the major source of cadmium [1-4]. Cadmium has many toxic effects and damages various organs such as liver, lung, kidney, testis and bones [5]. Oxidative stress is an important mechanism of cadmium toxicity and generally, antioxidants and plant flavonoids can directly scavenge molecular species of active oxygen and minimize the damaging effects of cadmium toxicity [6, 7]. Effective therapy for cadmium poisoning is difficult to achieve. Although there is no proven benefit, some clinicians recommend chelation therapy with $CaNa_2EDTA$ [8]. Silymarin, a flavonoid mixture with antioxidant property derived from the seed extract of *silybum marianum* (L.) Gaertn., prevented toxic effects of various substances such as carbon-tetrachloride, halothane, ethanol, acetaminophen, and galactosamine [9, 10]. Silymarin is a safe and nontoxic herbal drug used clinically in the treatment of liver disorders [11]. Thus, the present study was performed to investigate the effects of silymarin on the parameters indicative of cadmium-induced toxicity in rats.

Materials and Methods

Chemicals

Cadmium as cadmium chloride and silymarin were purchased from Fluka and Sigma respectively.

Animals

130 adult male Wistar rats weighting about 200-220 g were purchased from the central animal house of the Shaheed Sadooghii University Medical College (Yazd, Iran). The animals were housed under standard conditions of light and dark cycle (12 hr light and 12 hour dark) with free access to food and water one week before starting the study. The experiment protocol was approved by the medical college ethics committee.

Animal food preparation

Each rat consumed 30 g food daily so silymarin was mixed with food powder accordingly so that the rats took the required daily doses of silymarin. The pellets were prepared by a hand-operated machine.

Study protocol

130 adult male Wistar rats were divided into 13 groups each comprising 10 rats. 1

group as control group was not administered neither cadmium nor silymarin. Cadmium chloride (3mg/kg/week) was administered intraperitoneally to 12 groups for 6 weeks. The 12 groups were divided into two sets of 6 groups. In the first set, one group was kept as control and silymarin in the doses of 15, 30, 60, 120 and 240 mg/kg/week was administered orally to each group for 6 weeks. In the second set, one group was kept as control and the aforementioned doses of silymarin were administered orally to each group for 6 weeks after 6 weeks of cadmium administration. The doses of silymarin were based on a previous study [12].

Blood samples were taken after 6 weeks from the first set and after 12 weeks from the second set to determine serum AST (aspartate aminotransferase), ALT (alanine aminotransferase) and ALP (alkaline phosphatase) levels and catalase activity as parameters indicating cadmium-induced toxicity.

Determination of serum enzyme levels

The AST, ALT and ALP levels were determined through the Elisa method with kits obtained from the Pars Azmoon Company (Tehran, Iran).

Determination of serum catalase activity

Serum catalase activity was determined through enzymatic method based on H_2O_2 decomposition [13].

Statistical analyses

All values were expressed as mean± SD. One-way ANOVA followed by Tukey post-hoc test was used for data analyses. $P<0.05$ was considered as statistically significant.

Results

First set of groups

Cadmium increased significantly the AST and ALP levels in the control group compared with the normal (drug-naive) group (P=0.021 and P=0.049 respectively). Silymarin at the doses of 60 mg/kg and 120 mg/kg decreased the ALP levels significantly in the cadmium treated groups compared with the control group (P=0.047 and P=0.032 respectively) (Figure 1).

Cadmium significantly decreased catalase activity in the control group compared with the normal group (P=0.004). Silymarin at the doses of 30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg significantly increased catalase activity in the cadmium treated groups compared with the control group (P=0.0061, P=0.0052, P=0.0046, P=0.0041 respectively) (Figure 3).

Second set of groups

Cadmium increased the AST level significantly in the control group compared with the normal group (P=0.0001). Silymarin at the doses of 15 mg/kg, 30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg significantly decreased AST levels in the cadmium treated groups compared with the control group (P=0.001, P=0.0009, P=0.0005, P=0.0003 and P=0.0001 respectively) (Figure 2).

Cadmium significantly decreased serum catalase activity in the control group compared with the normal group (P=0.034). Silymarin at the doses of 30 mg/kg, 60 mg/kg and 120 mg/kg significantly increased catalase activity in the

cadmium treated groups compared with the control group (P=0.035, P=0.032 and P=0.030 respectively) (Figure 4).

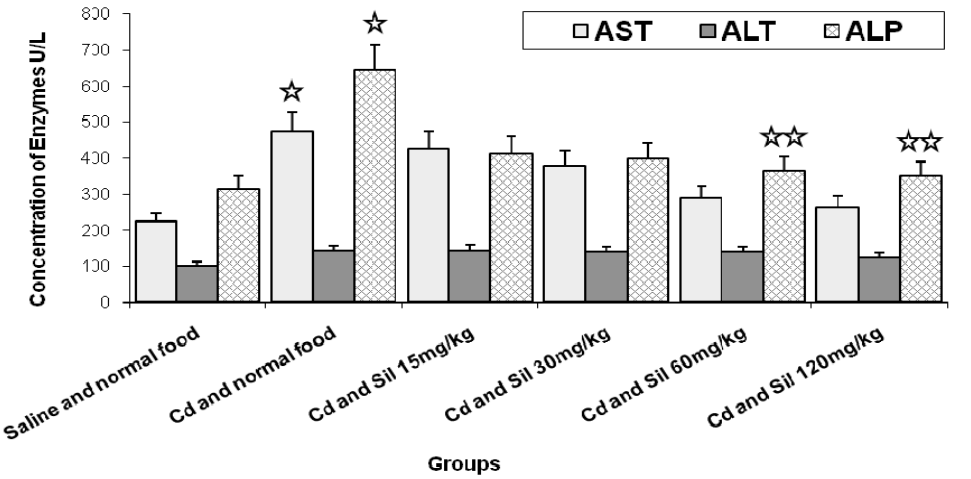

Figure 1- The effects of silymarin (sil.) on the serum enzyme levels in the first set of rat groups. The AST, ALT, and ALP levels in the silymarin treated groups were compared with the control group at the endpoint (One-way ANOVA and Tukey post-hoc tests). Each column represents the mean ± SD for 10 rats in each group.

☆ P<0.05 Control (cadmium treated) group compared with normal (drug-naive) group.

☆☆ P<0.05 Groups treated with cadmium and silymarin compared with control group.

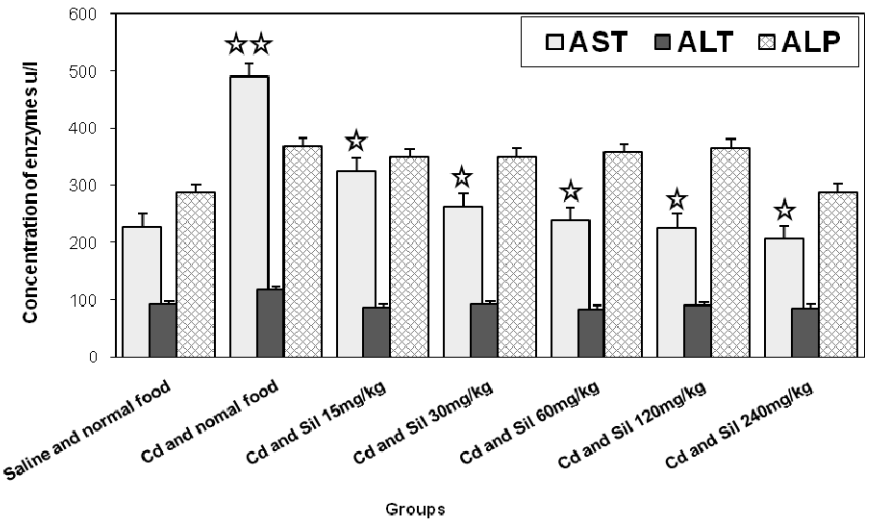

Figure 2- The effects of silymarin (sil.) on the serum enzyme levels in the second set of rat groups. The serum AST, ALT, and ALP levels in the silymarin treated groups were compared with the control group at the endpoint (One-way ANOVA and Tukey post-hoc tests). Each column represents the mean ± SD for 10 rats in each group.

☆☆ P<0.05 Control (cadmium treated) group compared with normal (drug-naive) group.

☆ P<0.05 Groups treated with cadmium and silymarin compared with control group.

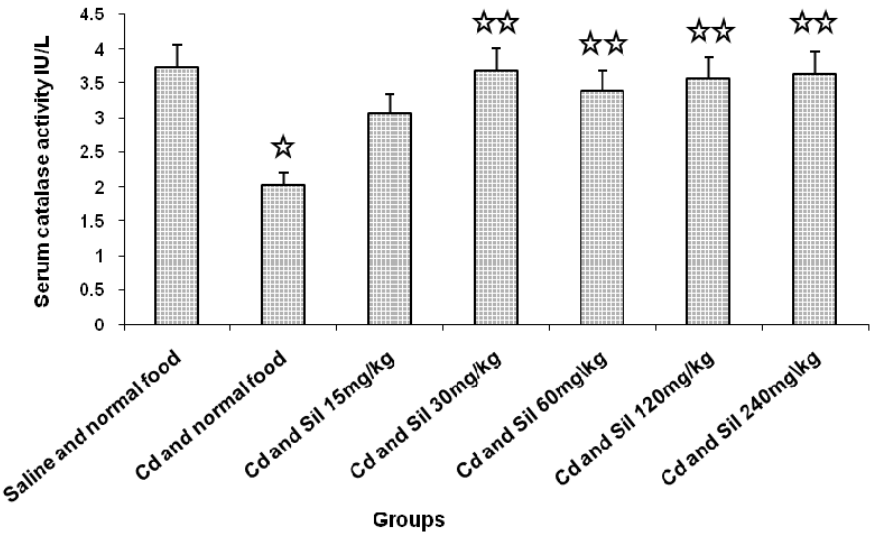

Figure 3 - The effects of silymarin (sil.) on catalase activity in the first set of rat groups. The serum catalase activity in the silymarin treated groups were compared with the control group at the endpoint (One-way ANOVA and Tukey post-hoc tests). Each column represents the mean ± SD for 10 rats in each group.

☆ P<0.05 Control (cadmium treated) group compared with normal (drug-naive) group.

☆ ☆ P<0.05 Groups treated with cadmium and silymarin compared with control group after 6 weeks.

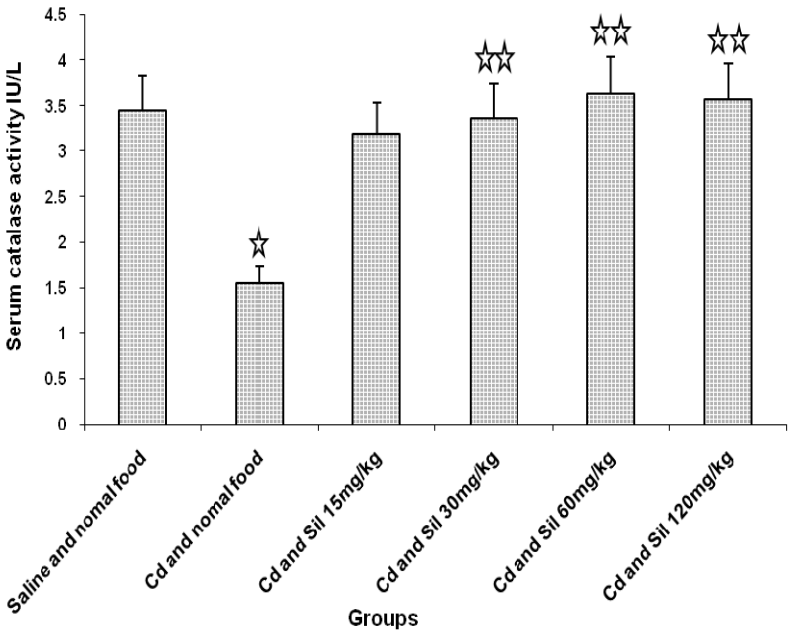

Figure 4- The effects of silymarin (sil.) on catalase activity in the second set of rat groups. The serum catalase activity in the silymarin treated groups were compared with the control group at the endpoint (One-way ANOVA and Tukey post-hoc tests). Each column represents the mean ± SD for 10 rats in each group.

☆P<0.05 Control (cadmium treated) group compared with control (drug-naive) group.

☆ ☆ P<0.05 Groups treated with cadmium and silymarin compared with control group after 12 weeks.

Discussion

The cadmium-induced alterations in the enzyme levels and activity indicate tissue damages. The results suggest that silymarin prevents and reverses the cadmium-induced toxicity probably through its antioxidative property. The results concur with a previous study in which silymarin protected against hepatotoxicity induced by antituberculosis drugs isoniazid, rifampin and pyrazinamide along with reduction of the blood AST, ALT and ALP levels as enzymes indicating liver function [14].

Although the exact mechanism of the effects of sylimarin on the cadmium-induced alterations of the enzyme levels and activity is unknown but anti-oxidative property of silymarin may be involved. The toxic manifestation of cadmium is caused primarily by imbalance between pro-oxidant and antioxidant homeostasis which is termed as “oxidative stress” [6]. Previous studies showed that natural dietary flavonoids and antioxidants may prevent and attenuate heavy metal induced biochemical alterations [7]. Silymarin is a mixture of flavonoids with powerful antioxidant properties. Its antioxidant property has been reported in several studies [9-11]. In the present study, the increase of serum catalase activity by silymarin is indicative of its antioxidative property in vivo. The present study shows that the effects of sylimarin on the cadmium-induced alterations of enzyme levels and activity may at least partly be due to its antioxidative property. It should be noted that a previous study reported that the cytoprotective effects of silymarin is mainly attributable to its antioxidant and free radical scavenging properties [15]. Another study also reported that the protective effect of silymarin against glutathione depletion induced by toxic agents is due to its antioxidant property [16]. Apart from the antioxidative effect, the membrane stabilizing and liver cell regenerating properties of silymarin may contribute to its hepato-protective effect [17, 10]. In conclusion, further studies into the effects of silymarin on the prevention and therapy of cadmium-induced toxicity and their mechanisms in laboratory animals and humans are recommended.

References

1. Moulis JM and Thevenod F. New perspectives in cadmium toxicity: an introduction. *Biometals* 2010; 23: 763 - 8.

2. Brzoska MM, Moniuszko-Jakoniuk J, Pilat-Marcinkiewicz B and Sawicki B. Liver and Kidney function and histology in rats exposed to cadmium and ethanol. *Alcohol. Alcohol.* 2003; 38: 2 - 10.

3. Fay RM and Mumtaz MM. Development of a priority list of chemical mixtures occurring at 1188 hazardous waste sites, using the HazDat database. *Food Chem. Toxicol.* 1996; 34: 1163 - 5.

4. Jawdat AA, Shah SA, Jaafar MH and Jadoo SA. Urinary cadmium concentration and its risk factors among adults in Tanjung

Karang, Selangor. *American-Eurasian J. Toxicol. Sci*. 2012; 4: 80 - 8.

5. Godt A, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A and Groneberg DA. The toxicity of cadmium and resulting hazards for human health. *J. Occup. Med. Toxicol*. 2006; 1: 22.

6. Siraj Basha P and Usha Rani A. Cadmium-induced antioxidant defense mechanism in fresh water teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicol. Environ. Saf*. 2003; 56: 218 - 21.

7. Michalak AB. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish J. Environ. Stud*. 2006:15: 523-30.

8. Brunton L, Parker K, Blumenthal D and Buxton L. Goodman & Gilman's Manual of Pharmacology and Therapeutics. McGraw-Hill Companies, Inc. USA. 2008, p: 1139.

9. Dixit N, Baboota S, Kohli K, Ahmad S and Ali J. Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches. *Indian J. Pharmacol.* 2007; 39: 172 - 9.

10. Chrungoo VJ, Singh K and Singh J. Silymarin mediated differential modulation of toxicity induced by paracetamol and D-galactosamine in freshly isolated rat hepatocytes. *Indian J. Exp. Biol*. 1997; 35: 611 -7.

11. Kroll DJ, Shaw HS and Oberlies N. Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies. *Integr. Cancer Ther*. 2007; 6: 110 - 9.

12. Radjabian T and Fallah Huseini H. Anti-hyperlipidemic and anti-atherosclerotic activities of silymarins from cultivated and wild plants of *silybum marianum* L. with different content of flavonolignans. *Iranian J. Pharmacol. Ther*. 2010; 9: 63 - 7.

13. Cohen G, Dembiee D and Marcus J. Measurement of catalase activity in tissue extracts. *Anal. Biochem*. 1970; 24: 30 - 8.

14. Eminzade S, Uras F and Izzettin FV. Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. *Nutr. Metab. (Lond.)*. 2008; 5: 18.

15. Soto C, Recoba R, Barron H, Alvarez C and Favari L. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol*. 2003; 136: 205 - 12.

16. Pradhan SC and Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. *Indian J. Med. Res*. 2006; 124: 491 - 504.

17. Muriel P and Mourelle M. Prevention by silymarin of membrane alterations in acute CCl4 liver damage. *J. Appl. Toxicol*. 1990; 10: 275 - 9.