Iranian Journal of Pharmaceutical Sciences
2019: 15 (4): 83-94
www.ijps.ir

Original Article

The Protective Effects of Saffron Stigma Alcoholic Extract against Vincristine Sulfate Drug-Induced Renal Toxicity in Rat

Asghar Beigi Harchegani[a], Milad Sohrabiyan[a], Hamid Bakhtiari Kaboutaraki[a], Hossein Shirvani[b], Alireza Shahriary[a*]

[a]Chemical Injuries Research Center, System Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran, [b]Exercise Physiology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran

Abstract

Vincristine (VCR) is a potential anti-cancer drug which is highly toxic for renal tissue. This study aims to evaluate the protective effect of alcoholic extract of saffron stigma against vincristine renal toxicity in male rats. A total of 50 rats were randomly divided into 10 groups. Different doses of VCR (0.25, 0.5 and 0.75mg/kg) and saffron (0.5 and 1mg/kg) were used to treat vincristine-induced renal toxicity in rats. Serum levels of creatinine (Cr), uric acid and blood urea nitrogen (BUN) were measured using specific kits at the end of the experimental period. Serum total antioxidant capacity (TAC) and malondialdehyde (MDA) values were measured using Ferric reducing antioxidant of power (FRAP) and Thiobarbituric acid reaction (TBAR) methods, respectively. Administration of VCR caused severe renal injury with significant increase in the levels of Cr, uric acid and BUN. VCR administration (at concentration of 0.75mg/kg) significantly increased the mean level of MDA (0.51±0.021 nmol/ml; $p<0.001$), while TAC value was declined significantly (243.17±16.24 µmol/l; $p<0.001$). These effects were dose-dependently. Treatment with saffron extract decreased the level of Cr, BUN, and MDA values in VCR-exposed rats with a significant enhancement in serum TAC content. This effect was notable for rats that received 1mg/kg plant extract. Administration of saffron, especially at higher concentration, can reduce VCR-induced renal toxicity, depletion of total antioxidant and lipid peroxidation, possibly due to its antioxidative properties.

Keywords: Vincristine, saffron extract; renal toxicity, TAC, MDA, oxidative stress

1. Introduction

Vincristine (VCR) is a natural alkaloid compound, which can be isolated from the leaves of *Catharanthus roseus* plant [1]. Recent studies have illustrated that VCR is a highly active cell cycle-dependent compound that binds to tubulin, leading microtubule depolymerization, metaphase arrest, and

Corresponding Authors: Alireza Shahriary, Chemical Injuries Research Center, System Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
Tel: (+98)21-82482502
E-Mail: Shahriary961@gmail.com
Cite this article as: Beigi Harchegani A, Sohrabiyan M, Bakhtiari Kaboutaraki H, Shirvani H, Shahriary A, The Protective Effects of Saffron Stigma Alcoholic Extract against Vincristine Sulfate Drug-Induced Renal Toxicity in Rat, 2019, 15 (4): 83-94.

subsequently apoptosis of mitotic cells [2-4]. Vincristine-spindle microtubules interaction changes structure and function of spindle dose-dependently [2]. Previous studies indicated that short-term exposure to VCR with low concentrations induces reversible mitotic arrest, inhibits chromosome segregation and results in abnormalities in polymerization of spindle microtubules [5]. In contrast, long-term exposure to VCR with higher concentrations is associated with disruption and total depolymerization of microtubule and eventually lethal cytotoxicity [6-8]. For this reason, VCR is now considered as a potential anticancer drug that can inhibit cancer cells proliferation. This compound has been extensively used for cancer therapy, especially for childhood and adult hematologic malignancies. Nevertheless, the antitumor property of VCR is depended on its concentration and duration of exposure [2].

Vincristine sulfate is a new formulation of VCR that is used widely for the chemotherapeutic management of a variety of pediatric malignancies [9]. Although it has a potent anti-tumor activity, its cytotoxicity effects on normal cells has limited its application. For example, recent studies have reported the cytotoxicity effect of VCR on various cell types such as hepatic, renal, pancreatic, and lymphocytes cells [10-13]. Therefore, it is essential to improve the therapeutic activity of VCR with increase in its dosage, whilst limiting free-drug-associated toxicity.

To reduce the cytotoxic effect of VCR, current study aims to evaluate the protective effects of saffron extract against vincristine sulfate-induced renal toxicity in rats. Saffron (*Crocus sativus*) is a potent and famous plant [14] that many studies showed the antioxidative and positive effects of its extract on human health [15-17]. A large number of studies have also considered saffron as a potential therapeutic drug in clinical trials [18, 19]. For example, the application of saffron extract in different types of diseases such as neuronal and cardiovascular disorders as well as cancer has been studied [20]. Recent evidence has illustrated that the health promoting property of saffron is mainly due to the presence of crocin [14]. It is a unique carotenoid compound with a potential antioxidant capacity that makes distinctive bright yellow color of the stigma [14].

Although several studies have considered positive effects of saffron on human health, less information is available about its renal toxicity effect after VCR treatment. We hypothesize that saffron administration may help maintain renal function by decreasing oxidative stress status and antioxidant depletion in rats exposed to VCR- sulfate. Therefore, the present study is designed to

investigate for the first time the effects of saffron extract on oxidative stress status and renal injuries in rats that received VCR-sulfate.

2. Materials and Methods

2.1. Saffron Preparation and Extraction Procedure

Saffron stigma was provided from the Novin Saffron Company, Mashhad, Iran. Saffron extract was isolated using maceration method. Briefly, about 50 g of stigmas were ground to powder and macerated in 1000 ml distilled water for 48 h. The mixture was subsequently filtered within 72 h and then concentrated under vacuum at room temperature. The extract yield was 50% w/w.

2.2. Study Design

In this experimental study, a total of 50 male Wistar rats (30-35 weeks of age) with a body weight of 200-250 g were provided from the laboratory animal research center of Tehran University of Medical Sciences, Iran. After one week adaptation with lab atmosphere, rats were randomly divided into 10 groups (n=5 for each group), including control, rats receiving 0.25 mg/kg (A group), 0.5 mg/kg (B group), 0.75 mg/kg (C group) vincristine sulfate, 0.25 mg/kg vincristine + 0.5 mg/kg saffron (D group), 0.5 mg/kg vincristine + 0.5 mg/kg saffron (E group), 0.75 mg/kg vincristine + 0.5 mg/kg saffron (F group), 0.25 mg/kg vincristine + 1mg/kg saffron (G group), 0.5 mg/kg vincristine + 1 mg/kg saffron (H group), and 0.75 mg/kg vincristine + 1 mg/kg saffron (I group) groups. Rats in each group were housed 3 per cage (30 × 15 × 15 cm) in a climate controlled room (ambient temperature of 22 ± 2°C, humidity 50 ± 5, and a 12:12 light/dark cycle) and had free access to food (10g/kg/day) and tab water. The study was approved by the animal care and use committee at the Islamic Azad University of Damghan.

Vincristine sulfate was injected intraperitoneally for 8 weeks. All injections were carried out at 10 am. After one week from the last injection, rats were anesthetized with diethyl ether and blood samples were provided from the aorta. Rats in D, E, F, G, H and I groups were subsequently treated orally with different concentrations of saffron extract for 8 weeks. Blood samples were then collected one week after the last administration of saffron for the assessment of liver enzymes. The normal control group was injected with sterile saline via the tail vein and with intragastrically administered distilled water. The study was approved by the animal care and use committee at the Baqiyatallah University of Medical Sciences.

2.3. Biochemical Analysis

Serum samples were isolated by centrifugation at 3000 rpm for 10 min for the assessment of Uric acid, BUN and Creatinine. The level of Uric acid (Cod: 23521), BUN (Cod: 23516) and Creatinine (Cod: 21502) was evaluated with the commercial ELISA kits provided from BioSystems Company. An Auto Analyzer apparatus (Roche Hitachi 911 and 912 models) was applied for the assessment of these renal biomarkers.

2.4. Total Antioxidant Capacity (TAC) Measurement

TAC value in serum of all samples was measured according to the Benzie method [21]. Briefly, 100 µl of serum sample was diluted 10-fold with distilled water and then immediately used for TAC assay. 1.5 ml of FRAP reagent (including Acetate buffer 300 mM, pH 3.6, TPTZ 10mM and Ferric chloride 20mM) was added to each tube and kept in water bath at 37^0C for 5 min. 50 µl of diluted serum sample was added to each tube, and again kept in water bath at 37^0C for 10 min. After 10 min, the absorbance of blank, standards (125, 250, 500 and 1000 µM/l FeSO4) and samples was assayed by spectrophotometer at 593 nm.

2.5. Malondialdehyde (MDA) Measurement

Malondialdehyde (MDA) content was assayed to estimate the lipid peroxidation in serum of rats. Thiobarbituric acid reactive substance (TBARS) method was applied for the evaluation of MDA content [22]. Briefly, 100 µl of the serum samples were mixed with 500 µl of Trichloroacetic acid (TCA), and then 10 µl of hydroxytoluene was added to the prepared solution and centrifuged at 3000 g for 10 min. 500 µl of the supernatant was removed from the solution, 400 ml of TBARS (144.14 g/mol; Merck, Germany) was added to it and the mixture was preserved at 95°C for one hour. Samples were stored and cooled down in a room temperature for 15 min. Then, they were centrifuged again at 4000 g for 10 min and the absorption of the supernatant was measured spectrophotometrically at 532 nm. MDA levels were calculated using the standard curve.

2.6. Statistical Analysis

All data are presented as means ±SD. The mean value of all parameters between different groups was compared using the One-Way ANOVA: Post Hoc-Tukey test. Data were analyzed using SPSS (version 19). A $p<0.05$ was considered as significant.

3. Results and Discussion

3.1. Results

Mean concentration of Creatinine, BUN and Uric acid in serum of all groups is shown in table 1. A significant difference was found in the mean concentration of all factors between groups ($p<0.0001$).

Administration of vincristine caused severe renal injury with considerable increase in the levels of Creatinine, BUN and Uric acid. The mean of Cr, BUN and uric acid in serum of rats received vincristine alone, especially at higher concentration (0.5 and 0.75 mg/kg), was significantly higher than that in the other groups ($p<0.0001$). This effect was dose-dependent, and rats that treated with dose of 0.75 mg/kg vincristine had the highest mean value of Cr (0.91±0.03 µg/kg; Figure 1), BUN (69.8±3.27 µg/kg; Figure 2) and uric acid (2.192±0.33 µg/kg; Figure 3) compared to the other groups ($p=0.00000$).

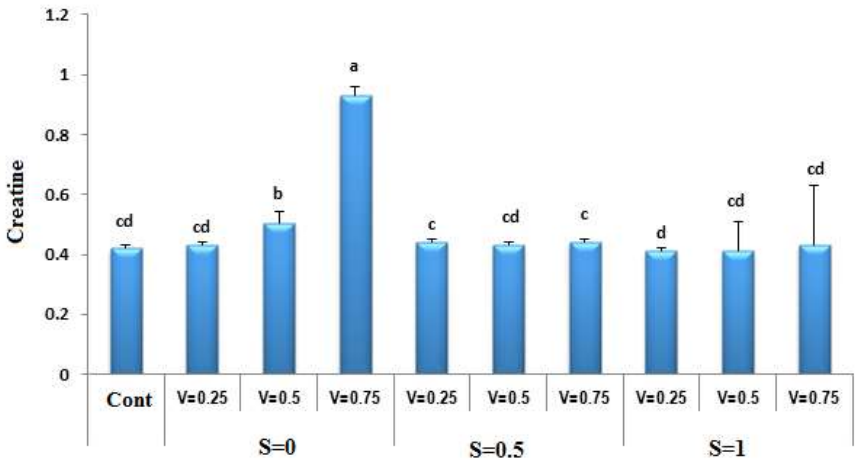

Figure 1. Comparison of the Creatinine levels between all groups (mean and SE). Cont: control; V: vincristine; S: stigma alcoholic extract.

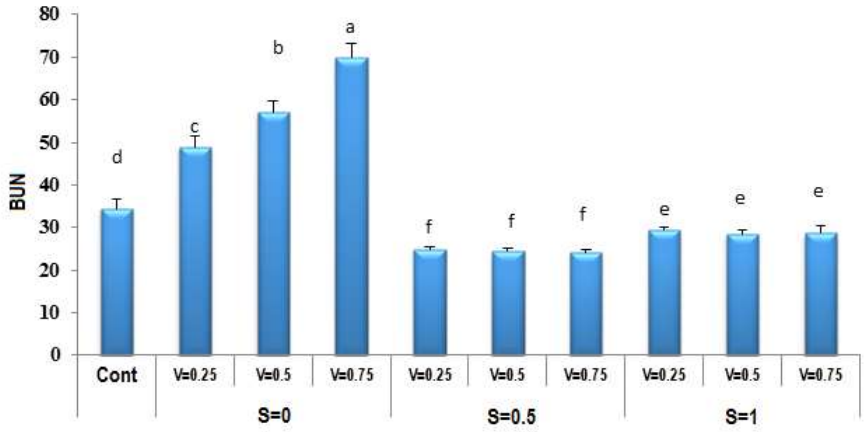

Figure 2. Comparison of the BUN levels between all groups (mean and SE). Cont: control; V: vincristine; S: stigma alcoholic extract.

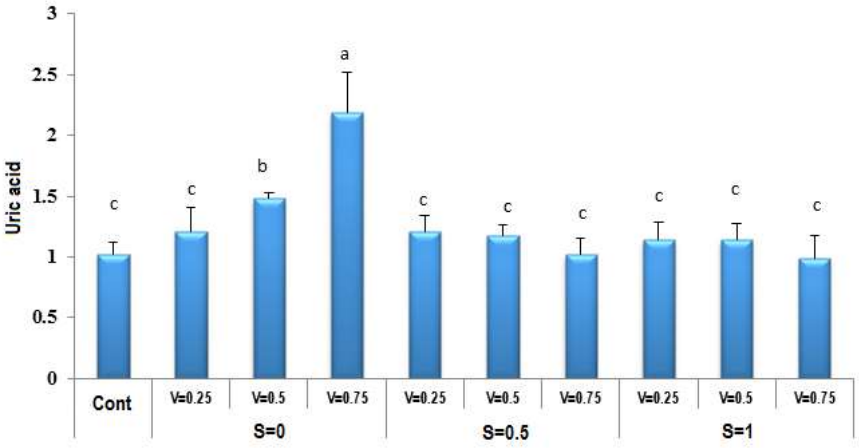

Figure 3. Comparison of the Uric acid levels between all groups (mean and SE). Cont: control; V: vincristine; S: stigma alcoholic extract.

Pre-treatment of rats with saffron extract reduced the level of these biomarkers to normal in a dose dependent manner. Pre-treatment at dosage of 1 mg/kg saffron extract showed a remarkable decrease. Rats that received higher concentrations of saffron had lower mean levels of Cr (Figure 1). The saffron treated group of E (V=0.25 mg/kg + S=1 mg/kg) had the lowest mean value of Cr (0.41±0.01 µg/kg). However, Cr level did not show much change in mixture V+S treated groups in comparison to control group. Similarly, treatment with saffron caused to significant reduction in the mean of BUN compared to the control and non-saffron groups (Figure 2; $p<0.001$).

Although treatment with vincristine increased the mean value of uric acid at higher levels (0.5 and 0.75 mg/kg), mixed treatment with saffron either at 0.5 or 1 mg/kg concentration normalized these changes (Figure 3). There was no significant difference in the mean concentration of uric acid between saffron treated groups at 0.5 and 1 mg/kg dosage.

VCR treatment decreased the TAC content (Figure 4), but increased the mean value of MDA (Figure 5) in a dose-dependent manner. Rats that received 0.75 mg/kg of VCR showed significantly ($p<0.001$) lower TAC concentration (243.17±16.24 µmol/l) compared to the other groups. In contrast, rats treated with 0.75 mg/kg of VCR demonstrated the highest mean level of MDA (0.51±0.021 nmol/ml) compared to the other groups (Figure 5). A trend was observed toward increased value of TAC and decreased level of MDA after treatments with saffron extract, especially at concentration of 1mg/kg.

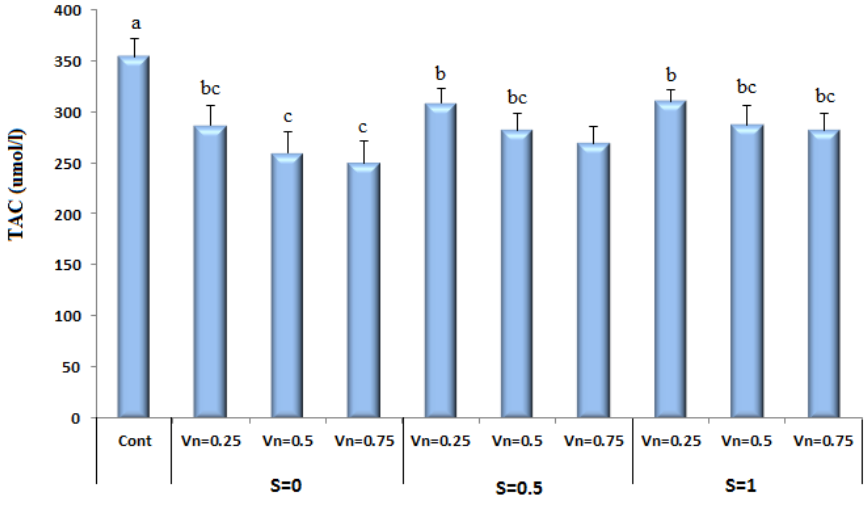

Figure 4. Comparison of TAC mean levels between all groups (mean and SE). Cont: control; V: vincristine; S: stigma alcoholic extract.

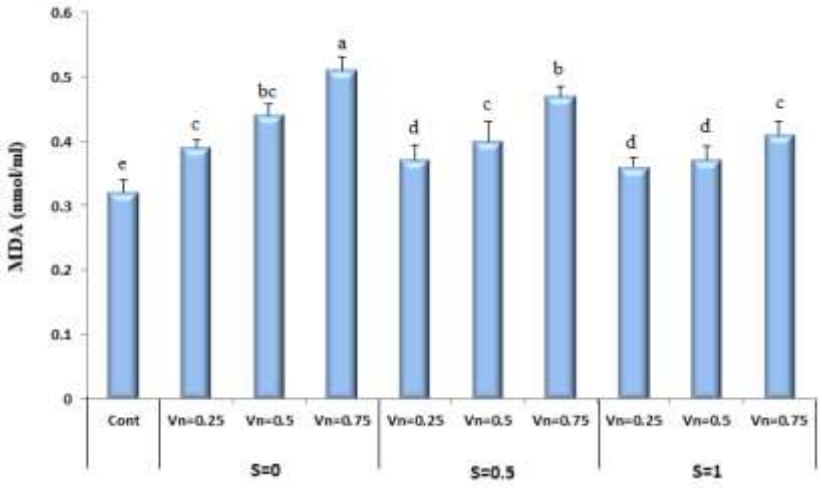

Figure 5. Comparison of MDA mean levels between all groups (mean and SE). Cont: control; V: vincristine; S: stigma alcoholic extract.

Table 1. The mean concentration of renal enzymes (U/I) in each group.

Groups	**Cr**	**BUN**	**Uric acid**
Control	0.42±0.01	34.40±2.19	1.016±0.10
Vn-0.25	0.43±0.01	49.00±2.35	1.212±0.02
Vn-0.5	0.50±0.04	57.2±2.39	1.476±0.05
Vn-0.75	0.91±0.03	69.8±3.27	2.192±0.33
Vn-0.25 + Z-0.5	0.44±0.01	24.92±0.28	1.21±0.13
Vn-0.25 + Z-1	0.41±0.01	29.48±0.69	1.14±0.15
Vn-0.5 + Z-0.5	0.42±0.01	24.44±0.78	1.18±0.08
Vn-0.5 + Z-1	0.41±0.01	28.52±1.03	1.144±0.13
Vn-0.75 + Z-0.5	0.44±0.01	24.34±0.55	1.02±0.13
Vn-0.75 + Z-1	0.43±0.02	28.72±1.79	0.988±0.19
P-value	<0.0001	<0.0001	<0.0001

3.2. Discussion

In this research, we evaluated the effects of 8 weeks treatment with saffron extract on vincristine sulfate-induced renal toxicity in male rats. Our data have revealed that administration of vincristine sulfate to the experimental rats, especially at higher dosage (0.5 and 0.75 mg/kg), leads to severe renal injury with considerable increase in the levels of serum creatinine, BUN and uric acid. Not only VCR declined the mean value of TAC, but also it enhanced the mean of MDA levels in serum of exposed rats. This agent induced renal toxicity in a dose-dependent manner. Interestingly, treatment with saffron extracts, especially at higher concentration (1mg/kg), decreased renal injury with considerable increase in TAC value and significant reduction in the mean level of MDA. These data suggest that saffron extract can prevent

VCR-induced renal toxicity through the inhibition of oxidative stress and antioxidant depletion.

Our findings are in agreement with other pervious research that indicated protective effects of saffron on several tissues such as liver, kidney, brain and heart. For example, Hosseinzadeh *et al*., considered the protective effect of aqueous saffron extract (with doses of 5, 20 and 80 mg/kg) on renal ischemia-reperfusion-induced oxidative damage in rats [23]. Their findings showed that saffron treatment protects kidney against renal ischemia-reperfusion through inhibition of oxidative stress. Mahmoudzadeh *et al*., investigated the anti-inflammatory and protective effects of saffron extract (5, 10 , and 20 mg/kg) against ischaemia/reperfusion-induced renal disturbances [24]. They showed that saffron extract can decrease the plasma creatinine concentration, lipid peroxidation biomarker level, TNF-α and intercellular adhesion molecule-1 expression and leukocyte infiltration in a dose-dependent manner [24]. In another study, Chahine *et al*., showed the cardioprotective effect of saffron extracts against acute doxorubicin toxicity in rabbits [25]. El-Beshbishy *et al*., indicated that saffron (with doses of 200 mg/kg) protects liver and brain against beryllium chloride ($BeCl_2$) toxicity in rats through reduction of oxidative stress and enhancing gene expression of antioxidant enzymes [26]. Shati *et al*., evaluated the effect of saffron extract on aluminum ($AlCl_3$)-induced hepatotoxicity [27]. Their findings revealed that saffron treatment minimizes the toxic effect of $AlCl_3$ with a significant improvement in serum cholesterol levels, triglycerides, GGT, ALT, AST, ALP and lipid peroxidation. The hepatoprotective effect of saffron extract was also illustrated against acetaminophen toxicity in male Wistar rats [28]. The protective effect of saffron extract (40 and 80 mg/kg for 8 weeks) on fatty liver tissue of high-fat diet induced obese rats was also considered in a previous research [29]. The results showed that saffron extract had dose-dependently alleviated levels of liver enzymes and histopathological changes in these rats. The authors suggested that saffron extract has hepatoprotective effect against non-alcoholic fatty liver disease and high-fat diet-induced liver damage [29]. The protective effect of saffron extract on liver cancer is also reported in several studies [30-32]. The administration of saffron with a dose of 20 mg/kg was found to be associated with lower levels of AST, ALT and bilirubin, with a significant higher concentration of total protein and albumin [28].

Although saffron therapy can protect kidney against vincristine sulfate, the mechanism by which saffron extracts improve renal injuries is not well-understood. Recent evidences have illustrated that overproduction of reactive oxygen species (ROS and oxidative stress (OS) can be considered as one of the main mechanisms in which vincristine sulfate leads to tissue injuries [33]. Inhibition of OS induced by vincristine sulfate and ROS is likely a significant mechanism by which saffron extract improves kidney injury. Interestingly, saffron treatment has been shown associated with a significant reduction

in malondialdehyde and protein carbonyls contents in rat liver and brain [26]. Pan *et al*., proposed that saffron can reduce hepatic injury through regulating protein oxidation [34]. Numerous studies have revealed that saffron extract has a potential antioxidative property and inhibits OS with considerably increase of different antioxidants [26, 35-37]. For example, Koul *et al*., reported that administration of saffron extract can reduce the content of lipid peroxidation with concomitant enhancement in antioxidants such as reduced glutathione (GSH) and activity of glutathione S-transferase (GST), glutathione peroxidase (GPX) and catalase (CAT) [38]. Saffron treatment has been also shown associated with significant increase in the survival of HCT116 cells, inhibition of ROS generation and lipid peroxidation, as well as higher activity of CAT and superoxide dismutase (SOD) [39]. The reduction in mitochondrial membrane potential, DNA fragmentation and Caspases activation was reported [39]. In another research, a significant reduction in the content of lipid peroxidation with a concomitant enhancement in the liver enzymatic (SOD, CAT, GST, GPx) and non-enzymatic antioxidants (GSH) was observed in animals received saffron [40]. A more recent study has demonstrated that saffron treatment reduces oxidative myocardial damage through antioxidant and antiapoptotic mechanisms [25]. Therefore, these findings support the idea that saffron may protect kidney against vincristine sulfate toxicity through inhibition of oxidative stress.

It is concluded that lipid peroxidation and antioxidants depletion is one of the major mechanisms by which VCR causes to severe renal injury. Administration of saffron, especially at higher concentration, can reduce VCR-induced renal toxicity, possibly due to its antioxidative properties.

Acknowledgments

We would also like to appreciate the staffs of the Biochemistry Department at Islamic Azad University, Damghan, Iran.

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