

ORIGINAL ARTICLE

The hepatoprotective and antioxidative effect of saffron stigma alcoholic extract against vincristine sulfate induced toxicity in rats

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ABSTRACT

Vincristine (VCR) is an important anti-cancer drug, which is highly toxic for the liver. This study aimed at evaluating the protective effect of alcoholic extract of saffron stigma against vincristine hepatotoxicity in the rat. A total number of 50 rats were randomly divided into 10 groups, including controls, rats receiving 0.25 mg/kg (A group), 0.5 mg/kg (B group), 0.75 mg/kg (C group) VCR, 0.25 mg/kg VCR + 0.5 mg/kg saffron (D group), 0.5 mg/kg VCR + 0.5 mg/kg saffron (E group), 0.75 mg/kg VCR + 0.5 mg/kg saffron (F group), 0.25 mg/kg VCR + 1mg/kg saffron (G group), 0.5 mg/kg VCR + 1 mg/kg saffron (H group), and 0.75 mg/kg VCR + 1 mg/kg saffron (I group) groups. Serum level of liver enzymes, including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and bilirubin 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, especially at the concentration of 0.75mg/kg, caused severe hepatic injury with significant increase in the levels of AST (582.0±39.45 UI), ALT (124.0±5.92 UI), ALP (939.8±89.8 UI) enzymes and bilirubin (0.17±0.008). VCR administration also significantly increased the serum MDA level (0.49±0.021 nmol/ml), while TAC value was declined significantly (241.27±18.27 μmol/l). These effects were dose-dependent. Treatment with saffron extract decreased the activity of liver enzymes and MDA values in hepatotoxic rats with a significant enhancement in serum TAC content. These effects were notable for rats that received 1mg/kg plant extract. Administration of saffron, especially at higher concentration, can reduce VCR-induced hepatotoxicity, antioxidant depletion and lipid peroxidation, presumably due to its antioxidative properties.

KEY WORDS: vincristine; saffron; hepatotoxicity; liver enzymes; oxidative stress

Introduction

Vincristine (VCR) is a natural alkaloid compound that can be extracted from the leaves of field grown *Catharanthus roseus* plant (Kumar *et al.*, 2013). Evidence revealed that it is a highly active cell cycle-dependent compound that targets tubulin, causing depolymerization of microtubule, M-phase arrest, and apoptosis in mitotic cells (van

Tellingen *et al.*, 1992; Gidding *et al.*, 1999; Silverman & Deitcher, 2013). The interaction between vincristine-spindle microtubules changes spindle structure and function in a dose-dependent manner (Silverman & Deitcher, 2013). At low concentrations and short-term exposure, it stimulates reversible mitotic arrest, prevents chromosome segregation and causes of some abnormalities in morphology or polymerization of spindle microtubules (Blajeski *et al.*, 2002). Higher VCR concentration and long-term exposure can be associated with disruption and total depolymerization of microtubule and subsequently lethal cytotoxicity (Jordan *et al.*, 1992; Takano *et al.*, 1993; Geyp *et al.*, 1996). For this reason, VCR is now considered a potential anticancer compound that has been widely used for therapeutic goals, particularly for childhood and

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adult hematologic malignancies. However, the antitumor activity of VCR is dependent on the concentration and duration of exposure and the number of cells transiting through mitosis during the period of drug exposure (Silverman & Deitcher, 2013).

Vincristine sulfate is a novel formulation of VCR used extensively in the chemotherapeutic management of a variety of pediatric malignancies (Thakur *et al.*, 2016). Despite its potent anti-tumor activity, it has cytotoxicity effects on normal cells. Many studies have reported the cytotoxicity effect of VCR on different cells such as hepatic, pancreatic, and lymphocyte cells (Nevalainen, 1975; Schrek & Stefani, 1976; el Saghir & Hawkins, 1984; Ogunc *et al.*, 2017). It is thus necessary to enhance the therapeutic activity of VCR to increase the VCR dose while limiting free-drug-associated toxicity.

To diminish the cytotoxic effect of VCR, this study aimed at considering the protective effects of saffron extract against vincristine sulfate-induced hepatotoxicity in the rat. Saffron, the dried stigma of the flowers of the saffron crocus (*Crocus sativus*), is now classified as a potent plant antioxidant (Mashmoul *et al.*, 2013). Many studies demonstrated the antioxidative and positive effects of saffron on human health (Nair *et al.*, 1995; Verma & Bordia, 1998; Samarghandian *et al.*, 2017). A great number of studies have also considered saffron as a potential therapeutic drug in clinical trials (Assimopoulou *et al.*, 2005; Kamalipour & Akhondzadeh, 2011). Thus the application of saffron extract in different types of diseases such as neuronal and cardiovascular disorders as well as cancer has been studied (He *et al.*, 2005). The health promoting properties of saffron are primarily due to the existence of a bioactive compound known as “crocin” (Mashmoul *et al.*, 2013). This is a unique carotenoid compound with a potential antioxidant capacity that makes the distinctive bright yellow color of the stigma (Mashmoul *et al.*, 2013).

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

Materials and methods

Plant material and extraction preparation

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

Study design

In this experimental study, 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. After a period of one week adaptation with lab environment, the rats were randomly allocated into 10 groups (n=5 for each group) including control. The rats received 0.25 mg/kg (A group), 0.5 mg/kg (B group), 0.75 mg/kg (C group) VCR, 0.25 mg/kg VCR + 0.5 mg/kg saffron (D group), 0.5 mg/kg VCR + 0.5 mg/kg saffron (E group), 0.75 mg/kg VCR + 0.5 mg/kg saffron (F group), 0.25 mg/kg VCR + 1 mg/kg saffron (G group), 0.5 mg/kg VCR + 1 mg/kg saffron (H group), and 0.75 mg/kg VCR + 1 mg/kg saffron (I group). In each group the rats 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 tap 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 a period of 8 weeks. All injections were carried out at 10 a.m. After one week from the last injection, the 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 for 8 weeks. Blood samples were collected one week after the last administration of saffron for measurement of liver enzymes. The normal control group was injected with sterile saline via the tail vein and with intragastrically administered distilled water.

Biochemical analysis

Serum samples were separated by centrifugation at 3000 rpm for 10 min for the assessment of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) enzymes and bilirubin. The activity of liver specific enzymes including AST (Cod: 11531), ALT (Cod: 11533) and ALP (Cod: 11593) 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 enzymes. Total bilirubin was measured using the Diazotized Sulfanilic method (Fossati *et al.*, 1989). Bilirubin reacts with diazotized sulfanilic acid and to form a blue azopigment that can be measured at 540 nm.

Malondialdehyde (MDA) measurement

Malondialdehyde (MDA) level was measured in order to estimate the lipid peroxidation in serum of rats. MDA content was assayed using thiobarbituric acid reactive substance (TBARS) method as described previously by Tavilani and colleagues (Tavilani *et al.*, 2005). 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 µl 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 at room temperature for 15 min. Then they were re-centrifuged at 4000×g for 10 min and the light absorption of the supernatant was determined by a spectrophotometer at the wavelength of 532 nm. Finally, MDA concentration was calculated, using the standard curve.

Total antioxidant capacity (TAC) measurement

Serum TAC was measured according to Benzie method (Benzie & Strain, 1996). Briefly, 100 µl of serum samples were 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 10 mM and ferric chloride 20 mM) was added to each tube and kept in water bath at 37°C for 5 min. Then, 50 µl of diluted serum sample was added to each tube, and again kept in water bath at 37°C 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.

Statistical analysis

All data are reported as means ±SD. The mean of all parameters between different groups was compared using the one-way ANOVA: *post-hoc* Tukey test. Data were analyzed using SPSS, version 19. The value of $p < 0.05$ was considered significant.

Results

The mean ± SD activity of AST, ALT, ALP enzymes and bilirubin contents in serum of all groups is shown in Figure 1. A significant difference was found in the mean values of all factors between groups ($p < 0.0001$).

VCR administration to the experimental rats caused severe hepatic injury with considerable increase in the levels of AST, ALT and ALP enzymes. The mean of these enzymes in serum of rats received VCR 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 treated with the dose of 0.75 mg/kg VCR had the highest mean value of AST, ALT and ALP enzymes compared to the other groups ($p = 0.00000$, Figures 1A–C).

Pre-treatment of rats with saffron extract reduced the level of these enzymes to normal. Mixed treatment with saffron reduced the mean of AST levels compared with the non-saffron groups (Figure 1A). The saffron treated group of F (V=0.75+S=0.5) showed the lowest mean value of AST (145.60±33.17 U/l). Although mixed treatment with saffron reduced the mean activity of ALT in C and I groups, we did not find a significant alteration in mean activity of this enzyme between A, B, D, E, G and H groups. Similarly, there were no significant difference in mean activity of ALP in A, B, D, E, F, G and I groups. However, combinational treatment with vincristine 0.5 mg/kg +

saffron 1 mg/kg (group H) declined the activity of ALP compared to the other groups.

The mean value of bilirubin in different groups can be seen in Figure 1D. Rats that received 0.75 mg/kg VCR and those in control groups showed the significantly highest mean concentration of bilirubin (0.17±0.008 and 0.19±0.02 U/l, respectively) compared to the other groups. Vincristine treatment increased the mean level of bilirubin in a dose-dependent manner. Saffron treatment, especially at higher concentration (1 mg/kg), declined significantly the mean of bilirubin in G, H and I groups.

VCR treatment decreased the TAC content (Figure 1E) and declined the MDA value (Figure 1F) in a dose-dependent manner. Rats that received 0.75 mg/kg of VCR showed significantly ($p < 0.001$) the lowest mean concentration of TAC (241.27±18.27 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 (0.49±0.021 nmol/ml) compared to the other groups (Figure 1F). A trend was observed toward increased value of TAC and decreased level of MDA after treatments with saffron extract, especially at the concentration of 1 mg/kg.

Discussion

In this study, we considered the effect of 8-week treatment with saffron extract on VCR sulfate-induced hepatotoxicity in male rats. Our data revealed that administration of VCR to the experimental rats led to severe hepatic injury with significant enhancement in the levels of AST, ALT and ALP enzymes as well as bilirubin. It not only decreased the mean value of TAC, it also increased the mean of MDA levels in serum of VCR exposed rats. This agent induced hepatotoxicity in a dose-dependent manner. Interestingly, treatment with saffron extracts, especially at higher concentration (1 mg/kg), decreased hepatic 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 hepatotoxicity through inhibition of oxidative stress and antioxidant depletion.

Our findings are in agreement with other research results. Several lines of studies indicated hepatoprotective effects of saffron. For example, Shati *et al.* considered the effect of saffron on aluminum (AlCl₃)-induced hepatotoxicity (Shati & Alamri, 2010). Their results showed that saffron treatment minimized the toxic effect of AlCl₃ with significant improvement in liver biochemical markers (cholesterol levels, triglycerides, GGT, ALT, AST and ALP) and lipid peroxidation. Another study investigated 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 (Mashmoul *et al.*, 2016). The results demonstrated that saffron extract dose-dependently alleviated the levels of liver enzymes and histopathological changes in these rats. The authors concluded that saffron extract has hepatoprotective effect against non-alcoholic fatty liver disease and high-fat diet-induced liver damage

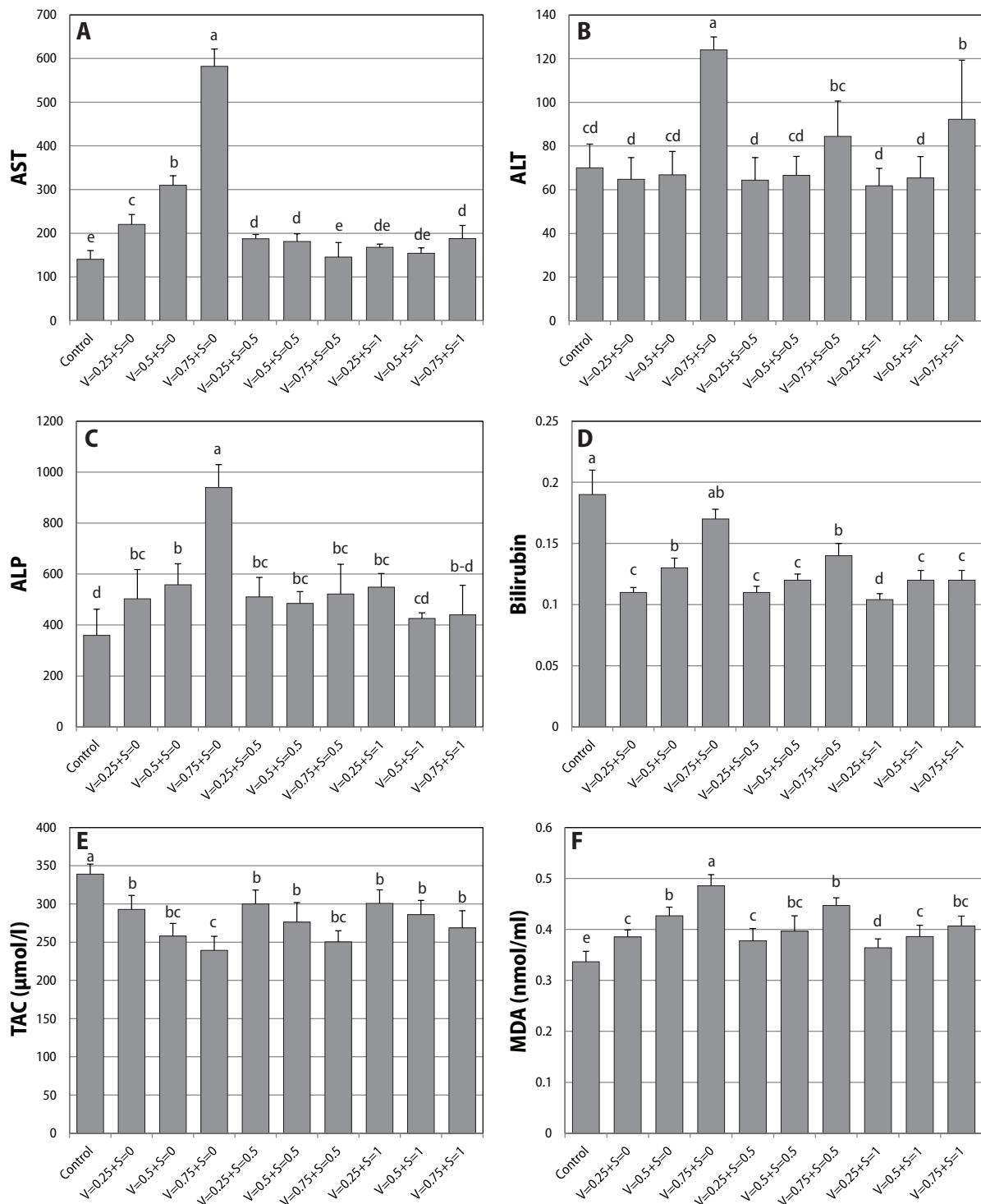


Figure 1. A: Comparison of AST activity between all groups (mean and SEM). There is a significant difference in mean AST activity between all groups ($p < 0.001$). Saffron treatment decreased the vincristine-induced AST activity especially at higher concentration. V: vincristine; S: saffron. **B:** Comparison of the ALT activity between all groups (mean and SEM). There is a significant difference in mean of ALT activity between all groups ($p < 0.001$). Saffron treatment decreased the vincristine-induced ALT activity. V: vincristine; S: saffron. **C:** Comparison of ALP activity between all groups (mean and SEM). There is a significant difference in mean ALP activity between all groups ($p < 0.001$). Saffron treatment decreased the vincristine-induced ALP activity especially at higher concentrations. V: vincristine; S: saffron. **D:** Comparison of the bilirubin levels between all groups (mean and SEM). There is a significant difference in mean bilirubin concentration between all groups ($p < 0.001$). Saffron treatment decreased the vincristine-induced bilirubin concentration. V: vincristine; S: saffron. **E:** Comparison of TAC mean levels between all groups (mean and SEM). There is a significant difference in mean TAC concentration between all groups ($p < 0.001$). Saffron treatment increased the TAC values in VCR treated rats. V: vincristine; S: saffron; TAC: total antioxidant capacity. **F:** Comparison of MDA mean levels between all groups (mean and SEM). There is a significant difference in mean MDA concentration between all groups ($p < 0.001$). Saffron treatment reduced the MDA values in VCR treated rats. V: vincristine; S: saffron; MDA: malondialdehyde.

(Mashmoul *et al.*, 2016). The protective effect of saffron on liver cancer was also considered in previous studies (Harrington, 2011; Amin *et al.*, 2016). In a clinical trial study, Hosseini *et al.* evaluated the effects of saffron capsules (50 mg, twice daily) on the response to treatment in patients suffering from liver metastases (Hosseini *et al.*, 2015). They suggested that saffron might be useful in these patients. The potential protective effect of saffron ethanol extract on hepatic ischemia-reperfusion injury was also reported in a previous study (Pan *et al.*, 2013). In another research, the hepatoprotective effect of saffron extract was evaluated against acetaminophen toxicity in male Wistar rats (Omidi *et al.*, 2014). 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 significantly higher concentration of total protein and albumin (Omidi *et al.*, 2014). These data are consistent with our findings, as we showed that saffron extract can reduce the mean value of ALT, AST, ALP and bilirubin. In addition to the hepatoprotective effects of saffron, numerous studies have reported its protective effect on different tissues, such as kidney (Hosseinzadeh *et al.*, 2005), brain (Berger *et al.*, 2011), skin (Das *et al.*, 2004), against a wide range of chemicals. Thus the protective effect of saffron extract against doxorubicin-induced acute cardiotoxicity in rabbit was also reported (Chahine *et al.*, 2014).

Although saffron treatments can protect the liver against vincristine sulfate, the mechanism in which saffron extracts improve these abnormalities is not well clear. Recent evidence has indicated that overproduction of free radicals and oxidative stress is one of the significant mechanisms in which vincristine sulfate causes tissue injuries (Martins *et al.*, 2011). Inhibition of oxidative stress (OS) induced by vincristine sulfate and reactive oxygen species (ROS) seem to be one of the mechanisms by which saffron extract improves liver injury. Interestingly, Pan *et al.* proposed that saffron can reduce hepatic injury through regulating protein oxidation (Pan *et al.*, 2013). Many studies have also revealed that saffron extract has an antioxidative property and prohibits OS with considerable increase of different antioxidants (Das *et al.*, 2010; El-Beshbishy *et al.*, 2012; Samarghandian *et al.*, 2014; Ghaffari *et al.*, 2015). For example, Koul and Abraham demonstrated that saffron pretreatment reduced the level of lipid peroxidation with concomitant increase in antioxidants such as glutathione content (GSH) and the activity of glutathione S-transferase (GST), glutathione peroxidase (GPX) and catalase (CAT) (Koul & Abraham, 2017). In an *in vitro* study, saffron treatment significantly increased the survival of HCT116 cells, inhibited the ROS generation, regulated the activity of CAT and superoxide dismutase (SOD) and reduced the lipid peroxidation biomarkers. A reduction in mitochondrial membrane potential, DNA fragmentation and caspases activation were also observed (Ben Salem *et al.*, 2016). In another research, the protective effect of saffron was considered on genotoxins-induced oxidative stress in Swiss albino mice (Premkumar *et al.*, 2003). The findings revealed 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) in saffron pretreated animals (Premkumar *et al.*, 2003). Recent evidence has also shown that administration of saffron extracts significantly reduced oxidative myocardial damage through antioxidant and antiapoptotic mechanisms (Chahine *et al.*, 2014). Mahmoudzadeh *et al.*, evaluated the anti-inflammatory and protective effects of saffron extract (5, 10, and 20 mg/kg) against ischemia/reperfusion-induced renal disturbances (Mahmoudzadeh *et al.*, 2017). They demonstrated that saffron extract can decrease the plasma creatinine concentration as well as lipid peroxidation biomarker level, TNF- α and intercellular adhesion molecule-1 expression and leukocyte infiltration in a dose-dependent manner (Mahmoudzadeh *et al.*, 2017). Therefore, these findings support the idea that saffron may protect liver against vincristine sulfate toxicity through inhibition of oxidative stress.

In summary, lipid peroxidation and total antioxidant depletion is one of the major mechanism by which VCR causes severe hepatic injury. Administration of saffron, especially at higher concentration, can reduce VCR-induced hepatotoxicity, possibly due to its antioxidative properties.

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