Antioxidant, anti-inflammatory, and anti-apoptotic effects of crocin against doxorubicin-induced myocardial toxicity in rats

Sara Asaad Abdul Kareem Aljumaily1 · Mehmet Demir2 · Hulya Elbe3 · Gurkan Yigitturk3 · Yasemin Bicer1 · Eyup Altinoz1

Received: 15 April 2021 / Accepted: 8 July 2021 / Published online: 28 July 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Doxorubicin (DOX) is a well-known chemotherapeutic drug for most malignancies including breast cancer and leukemia whilst the usage of DOX is limited owing to its cardiotoxicity. In the present study, we aimed to investigate the effects of crocin on doxorubicin-induced cardiotoxicity in rats. Forty rats were randomly divided into four groups: (a) control [received normal saline as a dose of 1 ml/kg by intraperitoneal injection (ip) for 15 days], (b) crocin (received crocin as a dose of 40 mg/kg/24h by ip for 15 days), (c) DOX (received DOX as a dose of 2 mg/kg/48h by ip in six injection, cumulative dose 12 mg/kg), and (d) DOX+ crocin (received DOX as a dose of 2 mg/kg/48h by ip in six injection, and crocin as a dose of 40 mg/kg/24h i.p for 15 days). As compared to the controls, the results showed that DOX administration caused significant increases in lipid indices [triglyceride (TG), low-density lipoproteins (LDL) (p<0.001), and very low-density lipoproteins (VLDL) (p<0.005)], oxidative stress parameters [malondialdehyde (MDA) and total oxidant status (TOS) (p<0.001)] and cardiac markers [creatine kinase-muscle/brain (CK-MB) and cardiac troponin I (cTnI) (p<0.001)]. Besides, significant decreases in antioxidant defense systems [glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and total antioxidant status (TAS) (p<0.001)] were observed. The present study also demonstrated that co-administration of crocin with DOX significantly ameliorated the lipid profile (p<0.005), cardiac markers (p<0.005), and oxidative stress indices (p<0.001) as compared to DOX group. Histopathologically, significant increase in the mean histopathological damage score (MHDS) was found in the DOX group as compared to the controls (p<0.001). In contrast, the administration of crocin with DOX alleviated MHDS in myocardium (p<0.001). Taken together, our results reveal that crocin might be a cardioprotective agent in DOX-treated patients for cancer.

Keywords Doxorubicin · crocin · apoptosis · oxidative stress · myocardial toxicity

Introduction

Doxorubicin (DOX), one of the anthracycline glycoside antibiotic, is a frequently used chemotherapeutic drug owing to treat several malignancies in patients, such as breast cancer, leukemia, and sarcoma by causing DNA intercalation and inhibiting the DNA replication (Migrino et al. 2008; Swain et al. 2003). The chief adverse effect of DOX treatment is dose-dependent cardiotoxicity that is a limiting factor for clinical usage of the drug during long-term administration in anticancer therapy (Ahmadian-Fard-Fini et al. 2018; Hong et al. 2002). The use of DOX in cancer treatment has greatly increased the survival rate of patients, but the incidence of causing heart failure (Lipshultz 2006) at DOX treatment is approximately 5% (Cardinale et al. 2015), even if the treatment dose is limited to 400 mg m⁻². For this reason, it is important to detect and treat patients with cardiotoxicity in order to reduce the rate of DOX-induced heart failure and improve the quality of life in DOX treatment. In addition, there are currently no imaging techniques or biochemical markers to diagnose DOX-induced heart failure at the onset of cardiac function decline, and there are no specific treatments to prevent DOX-induced cardiotoxicity (Hahn et al. 2014). There are
several molecular mechanisms for DOX-induced cardiotoxicity and all of them result in cardiomyocyte death (Kalyanaraman et al. 2002). Although the main mechanism of DOX-induced cardiotoxicity is unknown, DOX increases inflammation and oxidative stress in the heart tissue including lipid peroxidation, mitochondrial DNA damage, and apoptosis as well as deteriorate calcium homeostasis (Li et al. 2016; Octavia et al. 2012).

Increased both calcium and reactive oxygen species (ROS) in mitochondria leads to generate lipid peroxidation and the formation of oxidative injury and cell membrane of cardiomyocytes (Abushouk et al. 2019; Salavati-Niasari et al. 2009; Zhou et al. 2001a). Also, an increase oxidative stress which raised after DOX treatment contributes to the activation of apoptotic signaling pathways and leads to apoptotic cell death of myocytes (Octavia et al. 2012; Salehabadi et al. 2018). It seems that myocardial infarction, heart failure, and cardiac dysfunction are associated with myocardial apoptosis (Abbate et al. 2006; Abdel-Daim et al. 2017; Takemura et al. 2013). Due to the high effectiveness of DOX as a chemotherapeutic drug in many cancers, recent studies are associated with preventing and treating its adverse cardiac effects of DOX by using phytochemicals or drug along with DOX (Abushouk et al. 2017; Durdagi et al. 2021; Oner et al. 2019).

In traditional medicine, saffron (Crocus sativus L.) has been used for a long time as carminative, tonic, expectorant, and sedative (Mousavi et al. 2010). Also, it is reported that saffron is used to treat several desease including cardiovascular disorders, urological infections, and asthma (Tavakkol-Afshari et al. 2008). Crocin, one of the most common bioactive constituents of saffron, has many pharmacological properties, such as antioxidant, free radical scavenger (Mousavi et al. 2010), anticancer (Festuccia et al. 2014), anti-inflammatory (Hong and Yang 2013), and antiatherosclerotic and cardioprotective effects (Farkhondeh and Samarghandian 2014). The structure of crocin, water-soluble carotenoid pigment, is mono- and diglycosyl esters of a polyene dicarboxylic acid, named crocetin and responsible for the red color of saffron (Thushara et al. 2013). Some studies have reported that cardioprotective effects of crocin are associated with regulation of antioxidant enzymatic activities and cardiac markers (Hariri et al. 2010; Shen and Qian 2006). It has shown that crocin protects for cardiomyocytes against hypoxic damage by increasing the level of vascular endothelial growth factor (VEGF), which is an angiogenic protein (Wu et al. 2010).

Here, we aimed to assess the possible effects of crocin combined with DOX on cardiotoxicity, and the possible role of pathogenesis of DOX-induced cardiotoxicity, based on the specific cardiac biomarkers, biochemical parameters, oxidative stress markers, and histopathological and immunohistochemical evaluations in Wistar albino rats.

**Materials and methods**

**Animals**

Forty healthy adult male Wistar albino rats (10 weeks age, 225 ± 25 g) were purchased from Zonguldak Bulent Ecevit University Faculty of Medicine Experimental Animal Production and Research Center (ZBEUN-DEHAM). All rats were housed in a well-ventilated room with temperature- and humidity-controlled conditions (an ambient temperature range of 22 °C; relative humidity of 55–60%) in rat cages with a 12 h–12 h light–dark cycle (light from 08:00–20:00). The study was approved by the Experimental Animals Ethics Committee of Zonguldak Bulent Ecevit University, Faculty of Medicine (Protocol No: 2020-08-07/05). All experimental procedures were carried out in accordance with the Animal Ethics Committee Guidelines for the use of experimental animals. The experimental animals were allowed access to drinking water and standard rodent diet ad libitum.

**Study design**

Doxorubicin® 10 mg was purchased from Kocak Company (Istanbul, TURKEY) and crocin was obtained from Sigma Aldrich Corporation (St. Louis, Missouri, ABD). Forty Wistar rats were randomly aliquoted into four groups. At the beginning of the study, each experimental group consisted of ten animals. In group 1 (control), animals received normal saline (1 ml/kg) via intraperitoneal injection (i.p.) for 15 days. In group 2 (Crocin), animals received crocin (40 mg/kg) via i.p for 15 consecutive days (Razmarai et al. 2016c). In group 3 (DOX), animals received DOX (2 mg/kg) via i.p in six injection at 48-h intervals during the 12-day period (cumulative dose: 12 mg/kg) (Razmarai et al. 2016b). And in group 4 (DOX + crocin), animals received crocin (40 mg/kg) through 15 consecutive days (starting 4 days before first DOX administration) along with DOX teratment (with the same dose as the group mentioned above). Lyophilized DOX powder was prepared for i.p injection by dissolving with the solvent water. Crocin powder was dissolved by normal saline (0.9%). Throughout the study, all applications were carried out every day in the same time period.

**Blood and tissue collection**

At the sixteenth day of the experiment, all rats were anesthetized under ketamine/xylazine anesthesia. Then, the blood samples were taken into non-heparinized tubes from the abdominal vein. Following blood collection, all tubes were kept under room condition about 30 min for allowing to clot. The serum was obtained by centrifuging at 3000 rpm for 20 min and stored at −80°C until for determination of biochemical parameters. The rats were then killed by cervical decapitation.
and the heart tissues were removed immediately. The cardiac tissues were cleaned with ice-cold normal saline to remove excess blood then weighed. The tissues were divided into two equal parts. One of the parts was stored at −80°C until for measurement of biochemical parameters. The other part of cardiac tissue was fixed in 10% neutral buffered formalin for histological evaluations.

Biochemical analyzes

Preparation of samples

Rat heart tissues were removed from the freezer and rapidly weighed. Then the tissues were homogenized at 10,000 rpm for one min by an otomatic homogenizer (Bioprep-24 Homogenizer, Hangzhou Allsheng Instruments Co., Ltd., China) in a 10-fold volume ice-cold phosphate buffer (70 mM, pH 7.5). The homogenate was used for determination of MDA analysis. To obtain supernatant, the homogenates were centrifuged at +4°C and 3000 rpm for 10 min. The supernatants were used for determination of tissue biochemical parameters.

Assessment of oxidative stress markers

Heart tissue homogenate was used for assessment of lipid peroxidation by measuring cardiac MDA levels according to the method of Ohkawa et al. (Ohkawa et al. 1979). After adding 1% H3PO4 and 0.6% thiobarbituric acid with homogenate in test tubes, it was incubated in a boiling water bath for 45 min. Then, the samples were extracted by n-butanol and a pink colored product was formed. MDA level was calculated by reading this colored product in an ELISA reader at 535 nm. Results are given as nmol/gram wet tissue (gwt).

Heart tissue homogenates were centrifuged at 4000 rpm for 20 min to obtain the supernatant. These supernatants were used for other biochemical analyses. Cardiac superoxide dismutase (SOD) activity was determined with the method proposed by Sun et al. (Sun et al. 1988). In the experimental procedure, superoxide radicals are formed by the xanthine-xanthine oxidase reaction. These radicals cause the formation of blue colored formazan by reducing NBT (nitro blue tetrazolium) in the environment. Tissue SOD activities were calculated by measuring this blue formazan colour at 535 nm. Results were obtained as U/g protein.

Cardiac catalase (CAT) activity was determined by the method of Aebi (Aebi 1974). By mixing the tissue supernatants with phosphate buffer (pH: 7.5 mM) containing H2O2, the H2O2 in the medium was degraded into H2O and O2 by CAT activity. This degradation causes a decrease in absorbance at 240 nm. The decrease in absorbance was observed for 1 min to calculate enzyme activity. CAT activities were noted as K/g protein.

Reduced glutathione (GSH) content, one of the non-enzymatic antioxidant markers, was determined with the method illustrated by Ellman (Ellman 1959). After the tissue supernatants were deproteinized, the samples were mixed with 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) to form a yellow-green product. The GSH level was calculated by measuring the colored compound at 410 nm. Data were obtained as nanomol/gwt.

Cardiac protein content was assessed according to the Biuret method (Gornall et al. 1949) using bovine serum albumin (BSA) as standard and used for calculating the antioxidant enzyme's activities.

Total antioxidant status (TAS) of cardiac tissue was determined with the method proposed by Erel (Erel 2004). TAS content was studied according to the manufacturer’s instructions (Rel Assay Diagnostics, Gaziantep, TURKEY). Data were determined as mmol Trolox Equiv/L.

Total oxidant status (TOS) of cardiac tissue was measured according to the method of Erel (Erel 2005). TOS content was studied according to the manufacturer’s instructions (Rel Assay Diagnostics, Gaziantep, TURKEY). The results were obtained as μmol H2O2 equiv/L.

Assessment of cardiomyocytes damage

The serum samples were removed from freezer and kept under room condition for bring all samples to room temperature before use. The serum content of cardiac isoenzyme creatine kinase (CK-MB) and cardiac troponin I (cTn-I) was measured by using their respective ELISA kits for rats, according to the principle of kits (Elabscience, USA; Catalog number: E-EL-R1327 and E-EL-R1253, respectively).

Assessment of lipid profile

The serum content of lipid profile, including triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), was assessed by using their commercially available Architect c 1600 automatic analyzer kits by Abbott Laboratories (Illinois, USA).

Histological analysis

Histopathological evaluation

The heart tissues were fixed in 10% neutral buffered formalin for 48h. Tissues were washed in running water, and were dehydrated with increasing concentrations of ethanol (50%, 75%, 96%, and 100%). After dehydration, specimens were placed into xylene to obtain transparency and were embedded in paraffin. Paraffin blocks were cut at 5

 Springer
Results

Effects of crocin on cardiac oxidative stress markers and antioxidant parameters

Tables 1 and 2 present the average cardiac oxidative stress markers and tissue antioxidant contents. The values of cardiac MDA, antioxidant enzymes (SOD, CAT), GSH, TAS, and TOS were analyzed statistically. The levels of cardiac oxidative stress markers and tissue antioxidant contents showed no pathological changes between control and crocin groups. Statistical analysis showed that DOX treatment resulted in dramatically increases \( (p<0.001) \) in the cardiac levels of MDA and TOS relative to values measured within the control group. In addition, rats receiving DOX also revealed significant reductions \( (p<0.001) \) in cardiac GSH and TAS levels as well as the activities of antioxidant enzymes CAT and SOD as compared to levels measured within the control group. In contrast, the rats receiving DOX with crocin reversed significantly the effect of DOX administration on cardiac oxidative stress markers (MDA and TOS) \( (p<0.001) \) as compared to the DOX group. Similarly, treatment of DOX-intoxicated rats with crocin revealed significant increases in both antioxidant contents (GSH and TAS) \( (p<0.001) \) and the activities of antioxidant enzymes (CAT and SOD) \( (p<0.001) \) relative to values measured within the DOX group.

Effects of crocin on cardiotoxicity indices

Table 3 presents the comparison of mean serum cardiac CK-MB and troponin I values. The serum cardiotoxicity markers showed no pathological changes in the control and crocin groups. The data revealed that the levels of the serum cardiotoxicity indices (CK-MB and cTnI) dramatically increased \( (p<0.001) \) after DOX treatment relative to values measured within their control ones (control and crocin groups). On the other hand, the rats receiving DOX with crocin reversed significantly the effect of DOX treatment on cardiotoxicity indices CK-MB \( (p<0.001) \) and cTnI \( (p<0.001) \) as compared to the DOX group. On the other hand, the rats receiving crocin with DOX reversed significantly the effect of DOX treatment on cardiotoxicity indices \( (p<0.05 \) for CK-MB; \( p<0.001 \) for cTnI) as compared to the DOX group.

Effects of crocin on lipid profile

Table 4 presents the comparison of mean serum lipid parameters. The data demonstrated that rats receiving DOX revealed significant increases in serum TG and LDL levels \( (p<0.001) \) as well as VLDL levels \( (p<0.05) \) compared to the control group. In contrast, the rats receiving DOX revealed significant reduction in serum HDL levels \( (p<0.001) \) relative to values measured within the control group. On the other hand, rats

μm, mounted on slides, and stained with hematoxylin and eosin (H-E). The tissue sections were examined under light microscopy. The evaluated parameters for severity of cardiac damage were congestion, necrosis, infiltration, and loss of myofibrillar in 10 different fields for each section. For this analysis, cardiac damage was semiquantitatively graded as absent (0), mild (1), moderate (2), and severe (3), for each criterion. The maximum damage score was 12. All sections were examined using a Nikon Eclipse 80i light microscope and Nikon Image Analysis system.

Immunohistochemical (IHC) evaluation

For immunohistochemical analysis, sections were mounted on polylysine coated slides. After rehydrating, samples were transferred to citrate buffer (pH 7.6) and heated in a microwave oven for 20 min. After cooling for 20 min at room temperature, the sections were washed with phosphate buffered saline (PBS). Then, sections were kept in 0.3% H\(_2\)O\(_2\) for 7 min and afterward washed with PBS. Sections were incubated with an anti-TNF-α (1:100, bs-2081R, Bioss, China) and anti-caspase-3 (1:100, bs-0081R, Bioss, China) for 60 min. They then were rinsed in PBS and incubated with biotinylated goat antipolyvalent for 10 min and streptavidin peroxidase (SHP 125, ScyTek Laboratories, ABD) for 10 min at room temperature. Staining was completed with chromogen + substrate for 15 min, and slides were counterstained with Mayer’s hematoxylin (M06002, Bio-optica, ITALY) for 1 min, rinsed in tap water, and dehydrated. The antibody was used according to the manufacturer’s instructions. Staining for anti-TNF-α and anti-caspase-3 was identified by a brown color. The relative intensity of TNF-α and caspase-3 immunostaining was scored as follows: 0–5% (+), 6–20% (++, 21–40% (+++), 41–60% (++++), 61–80% (+++++), and 81–100% (++++++). All sections were examined using a Nikon Eclipse 80i light microscope and Nikon Image Analysis system.

Statistical analysis

Statistical analysis was carried out using the SPSS for Windows version 14.0 (SPSS Inc., Chicago, Ill., USA) statistical program. All data are expressed as arithmetic mean ± SE. Normality for continued variables in groups were determined by the Shapiro Wilk test. The variables did not show normal distribution \( (p<0.05) \). Kruskal-Wallis and Mann-Whitney \( U \) tests were used for comparison of variables among the studied groups. \( p<0.05 \) was regarded as significant.
receiving DOX with crocin decreased in serum TG and VLDL levels (p<0.001) as well as LDL (p<0.05) as compared to the DOX group. In addition, treatment of rats receiving DOX with crocin ameliorated serum HDL levels (p<0.05) relative to the DOX group.

**Histopathological findings**

The group 1 (control) and group 2 (crocin) were normal in histological appearance (Figure 1). There was no significant difference among these groups (p>0.05). Congestion, necrotic cells with picnotic nuclei, inflammatory cell infiltration, loss of myofibrillar, and cytoplasmic vacuolization were detected in DOX group (Figure 2a, b, and c). The mean histopathological damage score (MHDS) was 6.30±0.42 DOX group. Statistically significant increase in MHDS was found in the DOX group, when compared to the groups 1 and 2 (p<0.001, for all). The histopathological changes markedly regressed in DOX+crocin group (Figure 2d). The MHDS was 2.10±0.27 in the DOX+crocin group. When DOX group and DOX+crocin group were compared, statistically significant difference was detected (p<0.001). The MHDS for each group is given in Table 5.

**Immunohistochemical findings**

The expression of TNF-α in the heart tissues of the experimental groups was observed in the sarcoplasm, nuclei, and capillary vessels of cardiomyocytes. TNF-α expression is weak (0–5%) in groups 1 and 2. TNF-α expression is quite high in the DOX group (81–100%). In the DOX+crocin group, it is seen that the expression is moderate (41–60%) (Figure 3).

It was observed that the expression of caspase-3 in the heart tissues of the experimental groups was found in the sarcoplasm of cardiomyocytes and the tunica media layer (smooth muscles) of the vessels. Caspase-3 expression is weak (0–5%) in groups 1 and 2. In the DOX group, caspase-3 expression was moderate (41–60%). In the DOX+crocin group, it is seen that the expression is moderate to low (21–40%) (Figure 4). The intensity scores of TNF-α and caspase-3 immunostaining in cardiac tissue is given in Table 6.

**Discussion**

In the current study, we administered the cumulative dose of DOX (12 mg/kg) to observe cardiotoxicity during 2 weeks in line with previous studies (Babaei et al. 2020; Razmaraii et al. 2016b). The dose of DOX is the same as using in treatment of human cancers (Tarr et al. 2015). Based on previous evidence, DOX-induced cardiotoxicity is associated with dose-related and long-term administration of DOX (Takeamura and Fujiwara 2007). To our knowledge, the main mechanism of cardiotoxicity caused by DOX administration has not been

| Table. 1 Comparison of average tissue oxidant-antioxidant parameters |
|---------------------------------------------------------------|
| **Group** | **MDA (nmol/gwt)** | **GSH (nmol/gwt)** | **SOD (U/g protein)** | **CAT (K/g protein)** |
|-----------|------------------|------------------|-----------------|-----------------|
| Group 1: control | 395.36±26.88 | 483.50±1.99 | 80.79±3.06 | 8.46±0.45 |
| Group 2: crocin | 290.39±33.03 | 506.80±5.70 | 87.97±3.96 | 11.10±1.00 |
| Group 3: DOX | 1405.42±226.30 | 229.41±14.79 | 38.00±2.00 | 4.38±0.36 |
| Group 4: DOX + crocin | 495.10±5.91 | 345.28±13.78 | 57.62±3.05 | 7.09±0.62 |

Data are expressed as the arithmetic mean ± SE (n = 10). MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; gwt, gram wet tissue. Superscripts represent the statistically significant difference: a p<0.001 when compared to group 3 vs group 1 ve group 2, b p<0.001 when compared to group 4 vs group 2 and group 3, c p<0.05 when compared to group 4 vs group 2, d p<0.001 when compared to group 4 vs group 3

| Table. 2 Comparison of average tissue TAS and TOS values |
|--------------------------------------------------|
| **Group** | **TAS (mmol Trolox eqv/L)** | **TOS (μmol H202 Equiv./L)** |
|-----------|--------------------------|------------------|
| Group 1: control | 1.27±0.09 | 7.48±0.22 |
| Group 2: crocin | 1.52±0.09 | 7.06±0.14 |
| Group 3: DOX | 0.67±0.08 | 10.91±0.65 |
| Group 4: DOX + crocin | 1.20±0.17 | 7.33±0.26 |

Data are expressed as the arithmetic mean ± SE (n = 10). TAS, total antioxidant status; TOS, total oxidant status. Superscripts represent the statistically significant difference: a p<0.001 when compared to group 3 vs group 1 and group 2, b p<0.05 when compared to group 4 vs group 2, c p<0.001 when compared to group 4 vs group 3
exactly known yet. However, many researchers examining doxorubicin’s cardiotoxic effects have reported that the overproduction of ROS (Ahmed et al. 2005; Berthiaume and Wallace 2007) and the stimulation of inflammation (Deepa and Varalakshmi 2005) are responsible for DOX-induced adverse effects on myocardium. Owing to its lower antioxidant capacity in myocardium, the heart tissue is thought to be the main target tissue for DOX-induced oxidative damage relative to other tissues (De Beer et al. 2001; Zhou et al. 2001b). Also, myocardium contains cardiolipin, which is considered to have an attractive for DOX, resulting in DOX accumulation in the heart mitochondria, disturbance of the respiratory chain, and stimulation of apoptotic pathways (Ascensão et al. 2005).

Documentation for this hypothesis about ROS as the primary cause of DOX-induced cardiotoxicity has been demonstrated by numerous studies in transgenic animals, with the expression of antioxidant enzymes playing important protective roles against myocardial damage as a result of DOX administration (Kang et al. 1997; Sun et al. 2001). The formation of ROS and cardiac oxidative stress have been demonstrated in Wistar rats receiving DOX (Oner et al. 2019). The increase in ROS production with DOX administration results in oxidative stress and is one of the major causes of myocardial damage (Berthiaume & Wallace 2007). In view of these facts, researchers have suggested trials on antioxidant therapy on DOX-induced toxicity. Antioxidants ameliorate DOX-caused oxidative damage by scavenging free radicals and the regulation of the activities of antioxidant enzymes without inhibiting DOX’s anticancer properties. According to all these evidence, we studied the cardioprotective effects of crocin by biochemically and histologically. Previous studies have reported that several antioxidants including coenzyme Q, N-acetylcysteine, vitamin E, and vitamin C have cardioprotective activities to prevent myocardial damage.

### Table 3

| Group          | CK-MB (pg/ml) | cTn-I (pg/ml) |
|----------------|---------------|---------------|
| Group 1: Control | 16.62±1.67    | 175.50±17.58  |
| Group 2: Crocin | 15.76±1.32    | 186.16±12.02  |
| Group 3: DOX   | 32.36±2.15a   | 354.89±16.20a |
| Group 4: DOX + Crocin | 20.80±0.66abc | 231.96±7.85abc |

Data are expressed as the arithmetic mean ± SE (n = 10). CK-MB, cardiac isoenzyme creatine kinase; cTn-I, cardiac troponin I

\( a p < 0.001 \) when compared to group 3 vs group 1 and group 2

\( b p < 0.05 \) when compared to group 4 vs group 2

\( c p < 0.001 \) when compared to group 4 vs group 3

### Table 4

| Group          | TG (mg/dl) | VLDL (mg/dl) | HDL (mg/dl) | LDL (mg/dl) |
|----------------|------------|--------------|-------------|-------------|
| Group 1: control | 55.40±3.44 | 10.40±0.44   | 45.82±2.05 | 8.31±0.85   |
| Group 2: crocin  | 41.50±1.98a | 8.21±0.33a   | 50.96±2.68 | 7.22±0.67   |
| Group 3: DOX     | 88.40±5.17bc | 16.50±1.35bc | 37.40±1.67bc | 16.06±1.04bc |
| Group 4: DOX + crocin | 49.70±4.21d | 10.04±0.84d | 44.46±1.95d | 12.46±0.92d |

Data are expressed as the arithmetic mean ± SE (n = 10)

\( a p < 0.05 \) when compared to group 2 vs group 1

\( b p < 0.001 \) when compared to group 3 vs group 1

\( c p < 0.001 \) when compared to group 3 vs group 2

\( d p < 0.05 \) when compared to group 4 vs group 3

\( e p < 0.05 \) when compared to group 3 vs group 1

\( f p < 0.1 \) when compared to group 3 vs group 1

\( g p < 0.05 \) when compared to group 4 vs group 3

\( h p < 0.05 \) when compared to group 4 vs group 1

\( i p < 0.001 \) when compared to group 4 vs group 2

\( j p < 0.001 \) when compared to group 4 vs group 2

### Table 5

| Groups          | Histopathological damage score |
|-----------------|--------------------------------|
| Group 1: control | 0.30±0.15                      |
| Group 2: crocin  | 0.50±0.16                      |
| Group 3: DOX     | 6.30±0.42a                     |
| Group 4: DOX + crocin | 2.10±0.27abc                 |

Data are expressed as the arithmetic mean ± SE (n = 10)

\( a p < 0.001 \) when compared to group 3 vs group 1 and group 2

\( b p < 0.001 \) when compared to group 4 vs group 3
damage induced by antracyclines in animals (Songbo et al. 2019; Zamorano et al. 2016). Recently, several studies have demonstrated that crocin can be used as a new therapeutic drug, owing to its anticancer (Fernández 2006; Konoshima et al. 1998) and antioxidant properties (Assimopoulou et al. 2005; Hosseinzadeh et al. 2005). Whereas the mechanism of crocin, a water soluble carotenoid of saffron, is not yet fully established, it has been recognized that the effect of crocin on pathways may be similar to well-known carotenoids (Pham et al. 2000). Therefore, it has been accepted that crocin is able to ameliorate intracellular oxidative stress by increasing the activities of the antioxidant enzymes (Rahaiee et al. 2015).

One of the end product of lipid peroxidation, MDA, is an indicator of oxidative stress induced by free radicals. DOX causes to increase ROS, impairs membrane function, and exposes cardiac dysfunction via myocardial apoptosis. Elevations in the levels of lipid peroxidation of the heart tissue by increasing MDA and TOS levels following DOX administration were determined in the present study. In addition, co-administration of crocin with DOX resulted in significant amelioration of DOX-induced lipid peroxidation in rat myocardium. Protection of the heart by strengthening the cardiac antioxidant defense system against DOX-induced cardiac injury caused by ROS formation plays an important role in protection against DOX-induced myocardial damage (Arafa et al. 2014; Oner et al. 2019). Previous studies reported that DOX application caused impairment in antioxidant defense such as SOD, CAT, and GSH (Qi et al. 2020; Sadek et al. 2021). As antioxidant enzymes, SOD and CAT scavenge superoxide anion and hydrogen peroxide. GSH, non-enzymatic antioxidant, is a tripeptide formed by the combination of glutamate, cysteine, and glycine. GSH cleans ROS from the body with its powerful antioxidant properties. Our data suggested that the activities of cardiac SOD and CAT as well as the

---

**Figure 1** The control and crocin groups were normal in histological appearance. a Group 1 (control), H-E; ×20. b Group 2 (crocin), H-E; ×20

---

**Figure 2** DOX group showed cardiac damage including (a) necrotic cells with picnotic nuclei (arrows), (b) congestion (asterisk), loss of myofibrillar, and cytoplasmic vacuolization (c) inflammatory cells infiltration (arrow). a Group 3 (DOX), H-E; ×20. b Group 3 (DOX), H-E; ×20. c Group 3 (DOX), H-E; ×10. The histopathological changes markedly regressed in the DOX+ Crocin group. d Group 4 (DOX+crocin), H-E; ×20
levels of TAS and GSH remarkably decreased in the heart tissue of rats receiving DOX. The results also revealed that co-administration of crocin with DOX ameliorated antioxidant defense including SOD, CAT, GSH, and TAS. As a potent antioxidant, crocin shows its possible mechanism by directly scavenging ROS (Hosseinzadeh et al. 2005) and upregulating antioxidant enzyme genes (Deng et al. 2018). However, the regulatory pathway is still unclear. More research is needed for the future. In line with our findings, previous studies showed that crocin elevates the antioxidant capacity in cardiac tissue in different conditions such as isoprenaline-induced myocardial fibrosis and arsenic

**Figure 3** The intensity of TNF-α immunostaining in cardiac tissue. Control and crocin groups were similar. In the DOX group, apoptosis was most evident. The intensity of TNF-α was reduced in DOX+crocin group. a Group 1 (control), b Group 2 (crocin), c Group 3 (DOX), d Group 4 (DOX+crocin); ×20

**Figure 4** The intensity of caspase-3 immunostaining in cardiac tissue. Control and crocin groups were similar. In the DOX group apoptosis was most evident. The intensity of caspase-3 was reduced in DOX+crocin group. a Group 1 (control), b group 2 (crocin), c group 3 (DOX), d group 4 (DOX+crocin); ×20
Li et al. (2018) reported that crocin decreased total cholesterol, hyperlipidemic properties. In line with our findings, Li et al. ing these biochemical indices because of its anti- and crocin might occur its cardioprotective effect by improv-

duction is associated with increases in the serum lipid profile
resulted in a significant improvement in serum lipid profiles. 
we demonstrated that co-administration of crocin with DOX
levels as well as decrease in HDL levels. On the other hand,
studies have shown the evidence that crocin have protective
effects against cardiovascular-related disorders including ath-
ersclerosis, hiperlipidemia, and cardiac dysfunction
(Alavizadeh and Hosseinzadeh 2014; Li et al. 2018). In this
study, we observed increases in triglyceride, LDL, and VLDL
levels as well as decrease in HDL levels. On the other hand,
we demonstrated that co-administration of crocin with DOX
resulted in a significant improvement in serum lipid profiles.
According to our results, heart damage caused by DOX in-
duction is associated with increases in the serum lipid profile
and crocin may occur its cardioprotective effect by improving
these biochemical indices because of its anti-
hyperlipidemic properties. In line with our findings, Li et al.
(Li et al. 2018) reported that crocin decreased total cholesterol,
 triglyceride, and LDL, and increased HDL in a coronary ath-
ersclerosis rat model. Also, Haybar et al. (Haybar et al. 2019)
demonstrated that DOX administration caused to increase in
total cholesterol, TG, and LDL, and decreased in HDL whilst
 gemfibrozil improved the lipid panel in DOX-treated rats.

Normally, CK-MB and cTn-I are located in the cytoplasm
of cardiomyocytes and pass through cardiomyocytes into the
systemic circulation following cell membrane injury
(Goudarzi et al. 2018). Thus, CK-MB and cTnI are cardiac
markers and accepted as an indicator to evaluate cardiac dys-
functions. In our study, DOX-induced myocardial toxicity by
the formation of ROS caused to elevate CK-MB and cTn-I
whilst co-administration of crocin with DOX resulted in a
significant improvement in elevated cardiac biomarkers.
There is an evidence showing that crocin exhibits cardioprotective impact by decreasing serum cardiac markers
(Elsherbiny et al. 2016). According to previous study, DOX-
induced myocardial toxicity is associated with oxidative stress
(Oner et al. 2019). It is well established that crocin could be
potent antioxidant agent through downregulation of apoptotic
and inflammatory pathways and/or scavenging free radicals
(Elsherbiny et al. 2016).

On the other hand, the evidence suggested that the other
mechanisms in the pathogenesis of DOX-induced cardiotoxicity except oxidative stress involve apoptosis and
inflammation (Sun et al. 2016). Numerous literatures indicate
that inflammation has a key role in DOX-induced myocardial
injury (Chularojmontri et al. 2013; Imam et al. 2018; Shaker
et al. 2018). Also, there are many documents confirming that
DOX triggers a series of inflammatory responses in
cardiomyocytes by upregulating the NF-kB and provides cas-
cading release of some pro-inflammatory cytokines, including
TNF-α and IL-1 (Abd El-Aziz et al. 2012). Recently, it has
been demonstrated that the intensive increase of pro-
flammatory cytokines in the heart tissue may be the patho-
logical basis of DOX-induced cardiovascular damage
(Pecoraro et al. 2016). In compliance with previous studies,
our results demonstrated significant increases in caspase-3 and
tumor necrosis factor alpha (TNF-α) reactivities in myocardium
after DOX injection (Dash et al. 2015; Ma et al. 2019).
Also, Shaker et al. (Shaker et al. 2018) reported that DOX
administration caused to increase in inflammatory markers
such as TNF-α and interleukin-1 beta (IL-1β) and apoptotic
markers such as caspase-3. Moreover, caspase-3, known as a
cell death protease, plays an important role in cell apoptosis
(Müller et al. 1998). Activated caspase-3 can alter cell mor-
phology and degrade DNA, ultimately triggering apoptosis in
the cell. DOX administration causes disruption of the respira-
tory chain function in the mitochondria of myocardial cells.
The elevation of ROS production results in a significant dis-
ruption of the permeability of the mitochondrial membrane.
These events ultimately trigger the apoptosis cascade reaction
of caspas in the cell (Oliveira et al. 2006). The elevation of
oxidative stress induced by DOX administration stimulates
certain signaling pathways involving caspase-3 activation,
resulting in myocardial cell apoptosis (Dash et al. 2015).
In line with the literature, we found that DOX toxicity caused
serious increases in TNF-α and caspase levels in myocardium.
In contrast, our results revealed that increased inflammatory
cytokines and apoptotic markers evoked by DOX induction
improved by crocin treatment because of its anti-inflammatory
and anti-apoptotic effectiveness. Moreover, these data are sup-
ported by our histopathological evaluation showing improve-
ment in the histological architecture of myocardium through
crocin treatment. Consistent with the results of the present
study, Razavi et al. (Razavi et al. 2013) showed that crocin
might have beneficial effects against diazinon-induced
cardiotoxicity through regulating signaling pathways associ-
ated with oxidative stress and cell apoptosis. Also, Chu et al.
(Chu et al. 2020) reported that there were significant decreases in TNF-α and IL-6 levels with different doses of crocin treatment in DOX toxicity.

Taken together, present study demonstrated that crocin protected rat myocardium against DOX-induced cardiotoxicity by modulating oxidative stress, reducing apoptosis, and inhibiting hiperlipidemia because of its antioxidant, anti-inflammatory, and antihyperlipidemic properties. Overall, we suggest that crocin may be a very good choice against DOX-induced myocardial damage. However, before moving into clinical practice in patients with DOX-induced cardiomyopathy, it is essential to better understand the mechanisms involved and the factors that govern the crocin therapy.

**Author contribution** Sara Asaad ABDULKAREEM ALJUMAILY and Yasemin Bicer studied biochemical analysis, Mehmet Demir designed the study and collected the tissues, Hulya Elbe and Gurkan Yigitturk performed the histological examination of the hearth tissues, and Eyup Altınoz designed the study and calculated the biochemical results, and was a major contributor in writing the manuscript.

**Funding** The research was supported by Karabuk University Scientifict Research Fund (TYL-2020-2298).

**Availability of data and materials** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethical approval and consent to participate** The study was approved by the Experimental Animals Ethics Committee of Zonguldak Bulent Ecevit University, Faculty of Medicine (Protocol No: 2020/04).

**Consent to publish** Not applicable

**Conflict of interest** The authors declare no competing interests.

**References**

Abbate A, Bussani R, Amin MS, Vetrovec GW, Baldi A (2006) Acute myocardial infarction and heart failure: role of apoptosis. Int J Biochem Cell Biol 38:1834–1840

Abd El-Aziz TA, Mohamed RH, Pasha HF, Abdel-Aziz HR (2012) Catechin protects against oxidative stress and inflammatory-mediated cardiotoxicity in adriamycin-treated rats. Clin Exp Med 12:233–240

Abdel-Daim MM, Khalifa HA, Ahmed AA (2017) Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Cancer Chemother Pharmacol 80:745–753

Abushouk AI, Ismail A, Salem AMA, Afifi AM, Abdel-Daim MM (2017) Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. Biomed Pharmacother 90:935–946

Abushouk AI, Salem AMA, Saad A, Afifi AM, Afify AY, Afify H, Salem HS, Ghanem E, Abdel-Daim MM (2019) Mesenchymal stem cell therapy for doxorubicin-induced cardiomyopathy: potential mechanisms, governing factors, and implications of the heart stem cell debate. Front Pharmacol 10:635

Aebi H (1974) Catalase. In: Bergmeyer. Methods in enzymatic analysis Academic Press Inc., New York, pp 673–686

Ahmadian-Fard-Fini S, Salavati-Niasari M, Ghanbari D (2018) Hydrothermal green synthesis of magnetic Fe3O4-carbon dots by lemon and grape fruit extracts and as a phospholiminescence sensor for detecting of E. coli bacteria. Spectrochim Acta A Mol Biomol Spectrosc 203:481–493

Ahmed HH, Mannaa F, Elmegeed GA, Doss SH (2005) Cardioprotective activity of melatonin and its novel synthesized derivatives on doxorubicin-induced cardiotoxicity. Bioorg Med Chem 13:1847–1857

Alavizadeh SH, Hosseinzadeh H (2014) Bioactivity assessment and toxicity of crocin: a comprehensive review. Food Chem Toxicol 64:65–80

Anafı MH, Mohammad NS, Attein HH, Abd-Elaziz HR (2014) Protective effect of resveratrol against doxorubicin-induced cardiac toxicity and fibrosis in male experimental rats. J Physiol Biochem 70:701–711

Ascenso A, Magalhães J, Soares JM, Ferreira R, Neuparth MJ, Marques F, Oliveira PJ, Duarte JA (2005) Moderate endurance training prevents doxorubicin-induced in vivo mitochondrialopathy and reduces the development of cardiac apoptosis. Am J Phys Heart Circ Phys 289:H722–H731

Assimopoulou A, Simakos Z, Papageorgiou V (2005) Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 19:997–1000

Babaei H, Razmanaii N, Assadmahs G, Mohajel Nayezi A, Azarmi Y, Mohammadnejad D, Azami A (2020) Ultrastructural and echocardiographic assessment of chronic doxorubicin-induced cardiotoxicity in rats. Archives of Razi Institute 75:55–62

Berthiaume J, Wallace KB (2007) Adriamycin-induced oxidative mitochondrial cardiotoxicity. Cell Biol Toxicol 23:15–25

Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, Civeili M, Lamantia G, Colombo N, Curiglano G (2015) Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation 131:1981–1988

Chu X, Zhang Y, Yue Y, Li Z, Shi J, Wang H, Chu L (2020) Crocin protects against cardiotoxicity induced by doxorubicin through TLR-2/NF-κB signal pathway in vivo and vitro. Int Immunopharmacol 84:105648

Chularojmontri L, Gerdpresart O, Wuttanapatayakul SK (2013) Pumemelo protects doxorubicin-induced cardiac cell death by reducing oxidative stress, modifying glutathione transferase expression, and preventing cellular senescence. Evid Based Complement Alternat Med 2013:1–9

Dash SK, Chattopadhyay S, Ghosh T, Dash SS, Tripathy S, Das B, Bag BG, Das D, Roy S (2015) Self-assembled betulinic acid protects doxorubicin induced apoptosis followed by reduction of ROS–TNF-α-caspase-3 activity. Biomed Pharmacother 72:144–157

De Beer EL, Bottone AE, Voest EE (2001) Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. Eur J Pharmacol 415:1–11

Deepa P, Varalakshmi P (2005) Biochemical evaluation of the inflammatory changes in cardiac, hepatic and renal tissues of adriamycin-administered rats and the modulatory role of exogenous heparin-derivative treatment. Chem Biol Interact 156:93–100

Deng M, Li D, Zhang Y, Zhou G, Liu W, Cao Y, Zhang W (2018) Protective effect of crocin on ultraviolet B-induced dermal fibroblast photoaging. Mol Med Rep 18:1439–1446
Durdagi G, Pehlivan DY, Oyar EO, Bahceci SA, Ozbek M (2021) Effects of melatonin and adrenomedullin in reducing the cardiotoxic effects of doxorubicin in rats. Cardiovasc Toxicol 21: 354–364
Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70–77
Elsherbeny NM, Salama MF, Said E, El-Sherbiny M, Al-Gayyar MM (2016) Crocin protects against doxorubicin-induced myocardial toxicity in rats through down-regulation of inflammatory and apoptotic pathways. Chem Biol Interact 247:39–48
Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37:277–285
Erel O (2005) A new automated colorimetric method for measuring total oxidative status. Clin Biochem 38:1103–1111
Farkhondeh T, Samarghandian S (2014) The effect of saffron (Crocus sativus L.) and its ingredients on the management of diabetes mellitus and dislipidemia. Afr J Pharm Pharmacol 8:541–549
Fernández J-A (2006) Anticancer properties of saffron, Crocus sativus Linn. Advances in phytomedicine 2:313–330
Festuccia C, Mancini A, Gravina GL, Scarsella L, Llorens S, Alonso GL, Tatone C, Di Cesare E, Jannini EA, Lenzi A (2014) Antitumor effects of saffron-derived carotenoids in prostate cancer cell models. Biomed Res Int 2014:1–12
Gormall AG, Bardawill CJ, David MM (1949) Determination of serum proteins by means of the biuret reaction. J Biol Chem 177:751–766
Goudarzi M, Fatemi I, Siahpoosh A, Sezavar SH, Mansouri E, Mehrzadi S, Aminzadeh A, Khodayar MJ, Hariri AT, Moallem SA, Mahmoudi M, Memar B, Hosseinzadeh H (2013) Cardioprotective effects of linalool against doxorubicin-induced cardiotoxicity in experimental rats. Cardiovasc Toxicol 18:337–345
Hahn VS, Lenihan DJ, Ky B (2014) Cancer therapy–induced cardiotoxicity: basic mechanisms and potential cardioprotective therapies. J Am Heart Assoc 3:e000665
Hariri AT, Moallem SA, Mahmoudi M, Memar B, Hosseinzadeh H (2010) Sub-acute effects of diazinon on biochemical indices and specific biomarkers in rats: protective effects of crocin and safranal. Food Chem Toxicol 48:2803–2808
Haybar H, Goudarzi M, Mehrzadi S, Aminzadeh A, Khodayar MJ, Kalantar M, Fatemi I (2019) Effect of gum arabic on cardiotoxicity induced by doxorubicin in male experimental rats. Biomed Pharmacother 109:530–535
Hong Y-J, Yang K-S (2013) Anti-inflammatory activities of crocin derivatives from processed Gardenia jasminoides. Arch Pharm Res 36:933–940
Hong YM, Kim HS, Yoon H-R (2002) Serum lipid and fatty acid profiles in adriamycin-treated rats after administration of L-carnitine. Pediatr Res 51:249–255
Hosseinzadeh H, Sadeghnia HR, Ziaee T, Danaee A (2018) Protective effect of aqueous saffron extract (Crocus sativus L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. J Pharm Pharmac 8:387–393
Iliskovic N, Singal PK (1997) Lipid lowering: an important factor in preventing adriamycin-induced heart failure. Am J Pathol 150:727
Imam F, Al-Harbi NO, Al-Harbi MM, Ansari MA, Al-Amsari AF, Ansari MN, Al-Anazi WA, Bahashwan S, Almutairi MM, Alshammari M (2016) Apremilast prevent doxorubicin-induced apoptosis and inflammation in heart through inhibition of oxidative stress mediated activation of NF-κB signaling pathways. Pharmacol Rep 70:993–1000
Kalyanaraman B, Joseph J, Kalivendi S, Wang S, Konorev E, Kotamraju S (2002) Doxorubicin-induced apoptosis: implications in cardiotoxicity. Mol Cell Biochem 234:119–124
Kang YJ, Chen Y, Yu A, Voss-McCowan M, Epstein PN (1997) Overexpression of metallothionein in the heart of transgenic mice suppresses doxorubicin cardio toxicity. J Clin Invest 100:1501–1506
Konoshima T, Takasaki M, Tokuda H, Morimoto S, Tanaka H, Kawata E, Xuan L, Saito H, Sugimura M, Molnar J (1998) Crocin and crocetin derivatives inhibit skin tumour promotion in mice. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 12: 400–404
Li DL, Wang ZV, Ding G, Tan W, Luo X, Cricolla A, Xie M, Jiang N, May H, Kryuchenko V (2016) Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification. Circulation 133:1668–1687
Li J, H-t L, Cao L, Mi Y-N, Li S, Cao Y-X (2018) Crocin alleviates coronary atherosclerosis via inhibiting lipid synthesis and inducing M2 macrophage polarization. Int Immunopharmacol 55:120–127
Liang Y, Zheng B, Li J, Shi J, Chu L, Han X, Chu X, Zhang X, Zhang J (2020) Crocin ameliorates arsenic trioxide-induced cardiotoxicity via Keap1-Nrf2/HO-1 pathway: reducing oxidative stress, inflammation, and apoptosis. Biomed Pharmacother 131:110713
Lipschultz SE (2006) Exposure to anthracyclines during childhood causes cardiac injury, Seminars in oncology. Elsevier, pp 8–14
Ma T, Khandare AD, Mukherjee-Khandare AA, Bodhankar SL (2019) Fisetin, a plant flavonoid ameliorates doxorubicin-induced cardiotoxicity in experimental rats: the decisive role of caspase-3, COX-II, cTN-I, iNOS and TNF-α. Mol Biol Rep 46:105–118
Migritno RQ, Aggarwal D, Konev O, Brahmbhatt T, Bright M, Kalyanaraman B (2008) Early detection of doxorubicin cardiomyopathy using two-dimensional strain echocardiography. Ultrasound Med Biol 34:208–214
Mousavi SH, Tayarani N, Parsaei H (2010) Protective effect of saffron extract and crocin on reactive oxygen species-mediated high glucose-induced toxicity in PC12 cells. Cell Mol Neurobiol 30:185–191
Müller I, Niethammer D, Bruchelt G (1998) Anthracycline-derived chemotherapeutics in apoptosis and free radical cytotoxicity. Int J Mol Med 1:491–495
Oktawa Y, Tocchetti CG, Gabrielsson KL, Janssens S, Crijns HJ, Moens AL (2012) Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol 52:1213–1225
Okhawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
Oliveira P, Santos M, Wallace K (2006) Doxorubicin-induced thiol-dependent alteration of cardiac mitochondrial permeability transition and respiration. Biochem Mosc 71:194–199
Onzer N, Alnooz E, Elbe H, Ekinici N (2019) The protective and therapeutic effects of linalool against doxorubicin-induced cardiotoxicity in Wistar albino rats. Hum Exp Toxicol 38:803–813
Pecoraro M, Del Pizzo M, Marzocco S, Sorrentino R, Ciccarelli M, Iacarino G, Pinto A, Popolo A (2016) Inflammatory mediators in a short-time mouse model of doxorubicin-induced cardiotoxicity. Toxicol Appl Pharmacol 293:44–52
Pham TQ, Cormier F, Farnworth E, Tong VH, Van Calsteren M-R (2000) Antioxidant properties of crocin from Gardenia jasminoides Ellis and study of the reactions of crocin with linoleic acid. J Ethnopharmacol 69:219–225
Qi W, Boliang W, Xiaoxi T, Guoqiang F, Jianbo X, Gang W (2020) Cardamomin protects against doxorubicin-induced cardiotoxicity in mice by restraining oxidative stress and inflammation associated with Nrf2 signaling. Biomed Pharmacother 122:109547
Rahaei S, Moini S, Hashemi M, Shojasoadati SA (2015) Evaluation of antioxidant activities of bioactive compounds and various extracts obtained from saffron (Crocus sativus L.): a review. J Food Sci Technol 52:1881–1888
Razavi BM, Hosseinzadeh H, Movassaghi AR, Imenshahidi M, Abnous K (2013) Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure. Chem Biol Interact 203:547–555
Razmaraei N, Babaei H, Nayebi AM, Asadnasab G, Helan JA, Azarni Y (2016a) Cardioprotective effect of phenytoin on doxorubicin-
induced cardiac toxicity in a rat model. J Cardiovasc Pharmacol 67: 237–245
Razmaraii N, Babaei H, Nayebi AM, Assadnassab G, Helan JA, Azarmi Y (2016b) Cardioprotective effect of grape seed extract on chronic doxorubicin-induced cardiac toxicity in Wistar rats. Advanced pharmaceutical bulletin 6:423–433
Razmaraii N, Babaei H, Nayebi AM, Assadnassab G, Helan JA, Azarmi Y (2016c) Crocin treatment prevents doxorubicin-induced cardiotoxicity in rats. Life Sci 157:145–151
Sadek KM, Mahmoud SF, Zeweil MF, Abouzed TK (2021) Proanthocyanidin alleviates doxorubicin-induced cardiac injury by inhibiting NF-κB pathway and modulating oxidative stress, cell cycle, and fibrogenesis. J Biochem Mol Toxicol 35:e22716
Salavati-Niasari M, Fereshteh Z, Davar F (2009) Synthesis of oleylamine capped copper nanocrystals via thermal reduction of a new precursor. Polyhedron 28:126–130
Salehabadi A, Salavati-Niasari M, Ghiyasiyan-Arani M (2018) Self-assembly of hydrogen storage materials based multi-walled carbon nanotubes (MWCNTs) and Dy3Fe5O12 (DFO) nanoparticles. J Alloys Compd 745:789–797
Shaker RA, Abboud SH, Assad HC, Hadi N (2018) Enoxaparin attenuates doxorubicin induced cardiotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. BMC Pharmacol Toxicol 19:1–10
Shen X-C, Qian Z-Y (2006) Effects of crocetin on antioxidant enzymatic activities in cardiac hypertrophy induced by norepinephrine in rats. Die Pharmazie-An International Journal of Pharmaceutical Sciences 61:348–352
Songbo M, Lang H, Xinyong C, Bin X, Ping Z, Liang S (2019) Oxidative stress injury in doxorubicin-induced cardiotoxicity. Toxicol Lett 307:41–48
Sun Y, Oberley LW, Li Y (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34:497–500
Sun X, Zhou Z, Kang YJ (2001) Attenuation of doxorubicin chronic toxicity in metallothionein-overexpressing transgenic mouse heart. Cancer Res 61:3382–3387
Sun Z, Yan B, Yu WY, Yao X, Ma X, Sheng G, Ma Q (2016) Vitexin attenuates acute doxorubicin cardiotoxicity in rats via the suppression of oxidative stress, inflammation and apoptosis and the activation of FOXO3a. Experimental and therapeutic medicine 12:1879–1884
Swain SM, Whaley FS, Ewer MS (2003) Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer: Interdisciplinary International Journal of the American Cancer Society 97:2869–2879
Takemura G, Fujiwara H (2007) Doxorubicin-induced cardiomyopathy: from the cardiotoxic mechanisms to management. Prog Cardiovasc Dis 49:330–352
Takemura G, Kanoh M, Minatoguchi S, Fujiwara H (2013) Cardiomyocyte apoptosis in the failing heart—a critical review from definition and classification of cell death. Int J Cardiol 167:2373–2386
Tarr A, Stoebbe S, Tuennemann J, Baka Z, Pfeiffer D, Varga A, Hagemondorf A (2015) Early detection of cardiotoxicity by 2D and 3D deformation imaging in patients receiving chemotherapy. Echo research and practice 2:81–88
Tavakkol-Afshari J, Brook A, Mousavi SH (2008) Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. Food Chem Toxicol 46:3443–3447
Thushara R, Hemshekhar M, Santhosh MS, Jnaneswari S, Nayaka S, Naveen S, Kemparaju K, Girish K (2013) Crocin, a dietary additive protects platelets from oxidative stress-induced apoptosis and inhibits platelet aggregation. Mol Cell Biochem 373:73–83
Wu Y, Pan R-R, Geng P (2010) The effect of Crocin against hypoxia damage of myocardial cell and its mechanism. Zhongguo ying yang sheng li xue za zhi= Zhongguo yingyong shenglixue zazhi= Chinese journal of applied physiology 26:453–457
Zamorano JL, Lancellotti P, Rodriguez Muñoz D, Aboyans V, Asteiguiano R, Galdersmi R, Habib G, Lenihan DJ, Lip GY, Lyon AR (2016) 2016 ESC position paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC committee for practice guidelines: the task force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur Heart J 37:2768–2801
Zhou S, Palmeira CM, Wallace KB (2001a) Doxorubicin-induced persistent oxidative stress to cardiac myocytes. Toxicol Lett 121:151–157
Zhou S, Starkov A, Froberg MK, Leino RL, Wallace KB (2001b) Cumulative and irreversible cardiac mitochondrial dysfunction induced by doxorubicin. Cancer Res 61:771–777

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.