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Mitochondria as a Target for the Cardioprotective Effects of Cydonia oblonga Mill. and Ficus carica L. in Doxorubicin-Induced Cardiotoxicity

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ABSTRACT

Background Quince (Cydonia oblonga Mill.) and fig (Ficus carica L.) exhibit a broad spectrum of pharmacological activities. Regarding the cardiotoxic effect of doxorubicin (DOX) is mediated mainly through mitochondrial oxidative stress and dysfunction; the present study evaluated the cardioprotective effects of the aqueous extracts of Cydonia oblonga Mill. fruit (ACO) and Ficus carica L. fruit (AFC) against DOX-induced cardiotoxicity.

Methods Cardiomyocytes toxicity was induced in male Sprague Dawley rats by intraperitoneal (ip) injections of 2.5 mg/kg DOX 3 times per week for a period of 2 weeks. After heart failure was induced in the rats, the animals were decapitated and their hearts were immediately removed. Then, the cardiac mitochondria were isolated by differential ultracentrifugation, and the protective effects of each particular extract on mitochondrial oxidative stress and dysfunction were determined.

Results ACO and AFC ameliorated mitochondrial dysfunction in the isolated mitochondria and prevented mitochondrial reactive oxygen species formation, membrane lipid peroxidation, mitochondrial swelling, mitochondrial membrane potential collapse (%ΔΨm), and cytochrome c release. Also, the extracts significantly increased reduced glutathione levels and succinate dehydrogenase activity.

Conclusion These results indicated that ACO and AFC have beneficial effects against DOX cardiotoxicity which mediated by attenuating mitochondrial dysfunction. Therefore, it can be suggested that quince and fig may increase the therapeutic index of DOX.

Introduction

Doxorubicin (DOX), a member of the anthracycline family, is one of the most-used antineoplastic drugs, being highly active against an extensive variety of malignancies, even at lower doses [1]. Unfortunately, cardiotoxicity of DOX is the main side effect in 10 % of treated patients, which can cause irreversible heart failure many years after the cessation of chemotherapy [2]. Several mechanisms are in-

volved in DOX-induced cardiotoxicity, including intracellular calcium dysregulation, defects in iron handling, and oxidative stress [3].

Accumulating evidence indicates that DOX increases the generation of mitochondrial reactive oxygen species (ROS) in cardiomyocytes and that the alteration of mitochondrial function is the principal mechanism of this cardiotoxic effect [4]. It has been also demonstrated that DOX-induced heart failure is mediated by the reduction/oxidation cycling of DOX to generate superoxide radicals that consequently trigger mitochondrial swelling, ultrastructural changes, and mitochondrial dysfunction. These events finally lead to cardiomyocyte cell death that is responsible for cardiac damage [5]. Considering that a major challenge in managing patients receiving DOX is to reduce the cardiotoxic effects, it has been suggested that the maintenance of mitochondrial function plays a key role in protecting against DOX-induced cardiotoxicity [6].

An important aspect of horticulture is the cultivation of plants for food, fiber, biofuel, medicine and other products used to sustain and enhance human life. Horticulture was the key development in the rise of sedentary human civilization, whereby farming of domesticated species created food surpluses that nurtured the development of civilization [7, 8]. Cydonia oblonga Mill. fruit, commonly referred to as quince, and Ficus carica L. fruit, generally referred to as fig, are plants that have been used in Iranian traditional medicine for the management of cardiovascular diseases [9]. Cydonia oblonga Mill. belongs to the Rosaceae family and has antihypertensive, antiproliferative, hypolipidaemic, hepatoprotective, and renoprotective effects [10–13]. Recently, we reported the preventive effect of quince fruit against hepatocellular carcinoma and against carbon tetrachloride-induced hepatotoxicity [14, 15]. Ficus carica L. is a member of the Moraceae family and is one of the first plants to be cultivated by humans. It has various pharmacological properties, including anticancer and anti-inflammatory [16]. However, the cardioprotective effects of Cydonia oblonga Mill. and Ficus carica L. fruits on cardiotoxicity induced by DOX have not yet been studied. Thus, this study evaluated the protective roles of the aqueous extracts of Cydonia oblonga Mill. fruit (ACO) and Ficus carica L. fruit (AFC) against DOX-induced cardiotoxicity. To this end, it was determined whether each particular extract improved mitochondrial function in mitochondria isolated from the hearts of DOX-treated rats.

Materials and Methods

Chemicals

DOX hydrochloride was obtained from ACCORD HEALTHCARE (France). 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), reduced glutathione (GSH), 2',7'-dichlorofluorescin diacetate (DCFH-DA), dithiobis-2-nitrobenzoic acid (DTNB), Tris-HCl, sulfuric acid, Tetramethoxypropane (TMP), and Rhodamine 123 (Rh 123) were purchased from Sigma- Aldrich Co. (Taufkrichen, Germany). All chemicals were of analytical, HPLC or the best pharmaceutical grades.

Plant materials, preparation of the extracts

Cydonia oblonga Mill. and Ficus carica L. fruits were collected from Shahriar, Alborz province, Iran and Estahban, Fars Province, Iran, respectively. The collected fruits were scientifically approved by the Department of Botany, Shahid Beheshti University (Voucher number: 8 054 for Cydonia oblonga Mill. and 8 072 for Ficus carica L., deposited in: Shahid Beheshti University Herbarium). The fresh fruits were cleaned and then dried in the shade at room temperature. Fruits were decocted in water for 30 min. Accordingly, the extract was filtered, concentrated to the desired level (honey-like viscosity), and stored at − 20 °C. The moisture level of the extract was determined by placing 2 g of the final extract in an oven at 60–65 °C for 72 h and then weighing it. Weight loss was used as a moisture indicator. The final extract contained 24 % water. These extracts were dissolved in distilled water at the desired concentrations just before use [17, 18]. Total phenolic contents were found to be 43.42 ± 1.18 and 13.57 ± 0.28 mg gallic acid equivalents (GAE) per gram of ACO and AFC (mg of GAE/g of plant extract), respectively. The given values are mean ± SD of 3 different determinations.

Determination of total polyphenol contents

Total polyphenol contents were determined by spectrophotometry, using gallic acid as the standard based on the Folin–Ciocalteu method as previously described (Pearson's correlation coefficient: $r^2 = 0.9906$) [19].

Animals

Male Sprague-Dawley rats weighing 200 to 250 g were housed in ventilated plastic cages over PWI 8–16 hardwood bedding. There were 12 air changes per h, 12 h light photoperiods, an environmental temperature of 21–23 °C, and a relative humidity of 50–60 %. The animals were fed a standard normal chow diet and given tap water ad libitum. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments were conducted according to the ethical standards and protocols approved by the Committee of Animal Experimentation of Zanjan University of Medical Sciences, Zanjan, Iran.

Experimental protocol and study design

60 rats were randomly selected for the control group, and the remaining 60 rats received DOX to induce heart failure. DOX was administrated intraperitoneally (i.p.) in 6 equal injections (each containing 2.5 mg/kg) on alternate days over a period of 2 weeks for a cumulative dose of 15 mg/kg [20]. At the end of this period, heart failure in the surviving rats was confirmed through serum Big-endothelin-1 levels evaluated using the Big Endothelin-1 ELISA kit as a marker of the degree of left ventricular dysfunction [21]. At the end of the experimental period, rats were sacrificed and their cardiac mitochondria were isolated by differential ultracentrifugation. In the first step, the functions of the cardiac mitochondria isolated from normal rats and heart failure rats were compared. Then, the protective effects of quince and fig fruit extracts on mitochondrial function were evaluated in mitochondria isolated from the heart of DOX-treated rats. We chose a wide range of concentrations for the ACO and AFC in our pilot study and their inhibitory effects against DOX-induced mitochondrial toxicity were evaluated (data not shown). By omitting non-effective, poorly effective or toxic concentrations, we selected the concentrations of 50 and 100 µg/ml for ACO and 100 and 200 µg/ml for AFC. The extracts were added to the mitochondria 20 min before the measurement of mitochondrial factors.

Isolation of cardiac mitochondria

Rat heart mitochondria were isolated according to the previously published protocols with some modifications [22]. Rats were anaesthetized with i.p. injections of ketamine (50 mg/kg) and xylazine (10 mg/kg). For each rat, the thoracic cavity was opened and the heart removed. The heart was carefully separated from its surrounding connective tissues and vessels, minced and washed in a cold MSE buffer (mannitol solution containing 0.225 M D-mannitol, 75 mM sucrose and 0.2 mM EDTA, pH = 7.4) sufficiently to remove blood (until the washing fluid became colorless). To isolate the mitochondria, the separated heart was homogenized in a glass homogenizer with a Teflon pestle using a tissue homogenizer for 1 min at 4 °C. The homogenates were centrifuged at 2 000 × g for 10 min, and then the obtained supernatants were centrifuged at 10 000 × g for 10 min. The final mitochondrial pellet was preserved in a special buffer relative to every experiment. Mitochondria were isolated freshly for each experiment and used within 4 h of isolation, and all steps were strictly worked on ice to guarantee the isolation of high quality mitochondrial preparations. The isolation of mitochondria was established by the measurement of succinate dehydrogenase (SDH) activity [23].

Determination of protein concentration

The concentration of mitochondrial protein was measured by the Coomassie blue protein-binding method using BSA as the standard [24]. To keep the uniformity of experimental conditions, the mitochondrial samples were used in the same concentration (500 µg protein/mL) in all experiments.

Determination of mitochondrial ROS formation

To determine the level of mitochondrial ROS generation, DCFH-DA was added to the isolated mitochondria. It penetrated mitochondria and was hydrolyzed to nonfluorescent dichlorofluorescin (DCFH). This compound which is nonfluorescent in turn reacted with ROS and forms highly fluorescent dichlorofluorescein (DCF), which effluxed the mitochondria. The fluorescence intensity of DCF in the medium was measured using a HITACHI fluorescence spectrophotometer. Excitation and emission wavelengths were 500 and 520 nm, respectively. The fluorescence intensity of the isolated cardiac mitochondria (500 µg/ml protein concentration) in respiratory buffer containing 10 mM Tris, 0.32 mM sucrose, 0.5 mM $MgCl_2$, 20 mM MOPS, 0.05 mM EGTA, 0.1 mM KH_2PO_4, and 5 mM sodium succinate and DCFH-DA (final concentration of 10 µM) was documented [25].

Mitochondrial reduced glutathione assay

The level of glutathione (GSH) in the heart mitochondrial fraction (500 µg protein/mL) was estimated by a spectrophotometric method using DTNB as the indicator of GSH [26]. The intensity of the yellow color produced in the samples was recorded at 412 nm with a UV spectrophotometer (Infinite M 200, TECAN). GSH content was expressed as µg/mg protein.

Measurement of mitochondrial lipid peroxidation

The amount of malondialdehyde (MDA) as the marker of lipid peroxidation was measured by reading the absorbance of the supernatant layer at 532 nm with an ELISA reader instrument (Infinite M 200, TECAN). TMP was used as the standard sample, and MDA content was expressed as µg/mg protein [27].

Succinate dehydrogenase activity assay

The activity of succinate dehydrogenase (mitochondrial complex II) was determined by measuring the reduction of MTT to formazan at 570 nm using an ELISA reader instrument (Infinite M 200, TECAN) [28].

Estimation of mitochondrial membrane potential (MMP)

In the present study, we used a rhodamine 123 exclusion assay to detect the membrane potential of cells due to its selective accumulation and quenching in mitochondria. Loss of the membrane potential will lead to release of the dye and, consequently, the fluorescence intensity [29]. Our data were shown as the percentage of MMP collapse (%ΔΨm) in all treated groups. $CaCl_2$ (50 mM), a known inducer of mitochondrial permeability transition (MPT) and MMP collapse was used as a positive control.

Mitochondrial swelling assessment

The swelling of mitochondria as a result of the transport of ions into the mitochondria was assessed through changes in light scattering using a spectrophotometer ELISA reader (Infinite M 200, TECAN) set at 540 nm. The absorbance of the samples was monitored for 1 h [28]. $CaCl_2$ (50 mM), a known inducer of MPT was used as a positive control.

Determination of cytochrome c release

Cytochrome c released from mitochondria was measured by rat/mouse cytochrome c ELISA Kit (Quantikine M., R&D Systems, Abingdon, UK) according to the manufacturer's instructions. This test employs the quantitative sandwich enzyme immunoassay technique and is designed to accurately quantify natural rat or mouse cytochrome c in cell lysates and subcellular fractions [30].

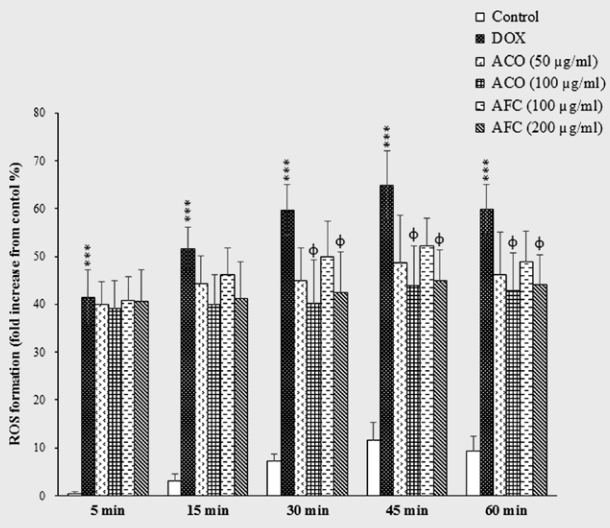
Statistical analysis

The homogeneity of variances was tested using Levene's test. The results were expressed as the mean ± SD of triplicate samples (n = 3) using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. The results with level of significance ($P < 0.05$) were regarded as significant.

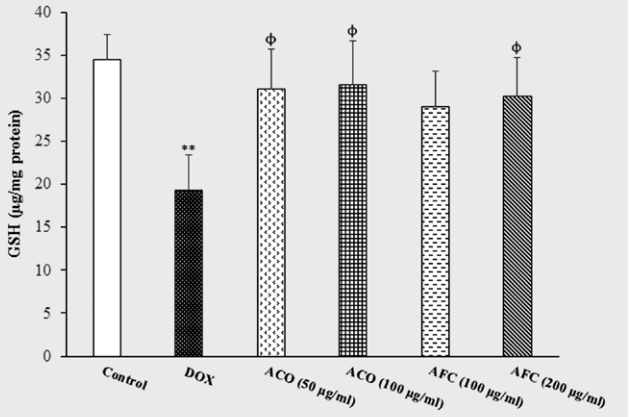
Results

Effects of ACO and AFC treatments on mitochondrial ROS formation

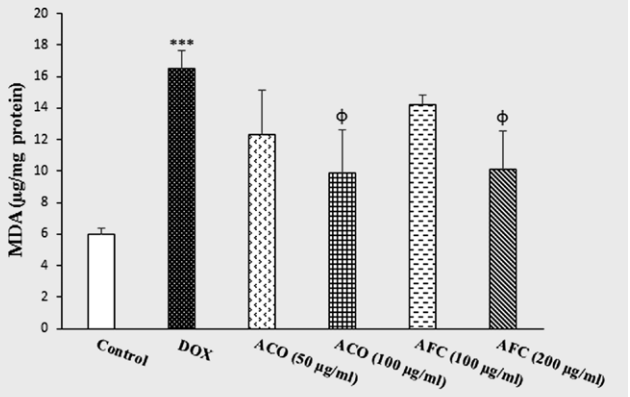
As shown in ▶ **Fig. 1**, ROS production determined by the oxidation of DCFH-DA to DCF was increased in the isolated heart mitochondria of DOX-induced heart failure rats compared with those of normal rats ($P < 0.001$). ACO and AFC, at concentrations of 100 µg/ml and 200 µg/ml, respectively, significantly ($P < 0.05$) prevented ROS formation in the mitochondria of DOX-treated rats after 30 min of incubation (▶ **Fig. 1**).



▶ **Fig. 1** Effects of ACO and AFC on mitochondrial ROS production in isolated heart mitochondria. DCF formation was expressed as fluorescent intensity units (FI units) and was shown as the percentage of fold increase from control group. Values are expressed as the mean ± SD of 3 separate experiments (n = 3). * * * P < 0.001 compared with isolated heart mitochondria of normal rats (Control group) in the same time; Φ P < 0.05 compared with isolated heart mitochondria of DOX-treated rats (DOX group).



▶ **Fig. 2** Effects of ACO and AFC on mitochondrial GSH content in isolated heart mitochondria. GSH levels were determined using DTNB reagent as indicator of reduced glutathione following 60 min of incubation. Values are expressed as the mean ± SD of 3 separate experiments (n = 3). * * P < 0.01 compared with isolated heart mitochondria of normal rats (Control group); Φ P < 0.05 compared with isolated heart mitochondria of DOX-treated rats (DOX group).



▶ **Fig. 3** Effects of ACO and AFC on lipid peroxidation in isolated heart mitochondria. MDA formation as the marker of lipid peroxidation was expressed as µg/mg protein following 60 min of incubation. Values are expressed as the mean ± SD of 3 separate experiments (n = 3). * * * P < 0.001 compared with isolated heart mitochondria of normal rats (Control group); Φ P < 0.05 compared with isolated heart mitochondria of DOX-induced heart failure rats (DOX group).

Effects of ACO and AFC treatments on mitochondrial GSH levels

GSH and its related antioxidant defense system prevent oxidative damage of lipids and other biomolecules caused by free radicals and have crucial roles in the maintenance of intracellular and intramitochondrial homeostasis. GSH levels were decreased in isolated heart mitochondria of DOX-treated rats compared with the control mitochondria (P < 0.01). Again, a decrease in mitochondrial GSH content was prevented by ACO at concentrations of 50 and 100 µg/ml and AFC (200 µg/ml) in the mitochondria of rats receiving DOX following 60 min of incubation (P < 0.05) (▶ **Fig. 2**).

Effects of ACO and AFC treatments on mitochondrial lipid peroxidation

Lipid peroxidation is usually one of the important consequences of ROS formation and oxidative stress in biological systems. The current results showed that lipid peroxidation also occurred in the mitochondria of DOX-induced heart failure rats, and a significantly (P < 0.001) increased amount of MDA was formed as compared with those of control group. MDA concentrations were decreased when the mitochondria were incubated with ACO and AFC at concentrations of 100 µg/ml and 200 µg/ml, respectively, following 60 min of incubation (P < 0.05) (▶ **Fig. 3**).

Effects of ACO and AFC treatments on complex II activity

Succinate dehydrogenase (complex II) is responsible for most cellular reduction and is the main site of MTT reduction in the mitochondrial fraction. The MTT assay is considered a succinate dehydrogenase inhibition assay. The activity of complex II showed a significant reduction (P < 0.01) in heart mitochondria of rats receiving DOX as determined by the formation of formazan. In addition, the pretreatment of mitochondria with ACO and AFC at concentrations of 100 µg/ml and 200 µg/ml, respectively, increased SDH activity following 60 min of incubation (P < 0.05) (▶ **Fig. 4**).

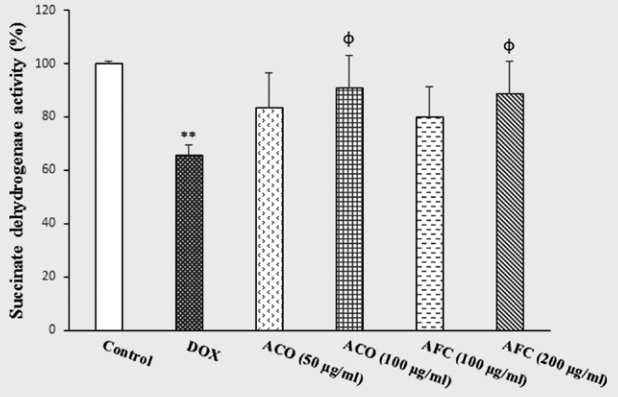
Effects of ACO and AFC treatments on MMP

MMP decline is the concluding marker of mitochondrial toxicity in all mitochondrial damaging pathways. As shown in ▶ **Table 1**, MMP (%ΔΨm) was decreased in isolated heart mitochondria of rats receiving DOX as compared with the control mitochondria (P < 0.001).

Elevated levels of %ΔΨm were prevented by ACO and AFC at the concentrations of 100 µg/ml and 200 µg/ml, respectively ($P<0.05$) (▶ **Table 1**).

Effects of ACO and AFC treatments on swelling

Mitochondrial swelling reflects the opening of mitochondrial ion channels and membrane pores which was evaluated through monitoring the changes in the absorbance of mitochondrial samples at 540 nm. Reduced absorbance is related to increase in mitochondrial swelling. The current results showed that there was significant decrease ($P<0.001$) in absorbance in the heart mitochondria of DOX-treated rats as compared with the control mitochondria. Similarly, ACO and AFC, at concentrations of 100 µg/ml and 200 µg/ml, respectively, prevented swelling in the mitochondria of DOX-treated rats ($P<0.001$) (▶ **Fig. 5**).



▶ **Fig. 4** Effects of ACO and AFC on succinate dehydrogenase (complex II) activity in isolated heart mitochondria. Succinate dehydrogenase activity was measured using MTT dye following 60 min of incubation. Values are expressed as the mean ± SD of 3 separate experiments (n = 3). * * $P<0.01$ compared with isolated heart mitochondria of normal rats (Control group); Φ $P<0.05$ compared with isolated heart mitochondria of DOX-induced heart failure rats (DOX group).

Effects of ACO and AFC treatment on cytochrome c release

The release of cytochrome c from mitochondria is the endpoint of mitochondrial toxicity which starts cell death signaling.Cytochrome c release occurred in the heart mitochondria of DOX-induced heart failure rats compared with the control mitochondria ($P<0.001$). This toxic event was prevented by ACO and AFC, at the concentrations of 100 µg/ml and 200 µg/ml, respectively following 60 min of incubation ($P<0.01$ for ACO and $P<0.05$ for AFC) (▶ **Fig. 6**).

Discussion

DOX is the most effective anticancer drug used in the treatment of various types of cancer. Unfortunately, its clinical utility is limited by the development of severe cardiotoxicity that results in cardiomyopathy and heart failure. So, many strategies have been tried to prevent or attenuate the cardiotoxic effects of DOX in cancer patients. However, the ability of strategies to prevent or ameliorate the cardiotoxicity has been limited and discovery of new agents for decreasing the most dangerous side effect of DOX is necessary [5, 31].

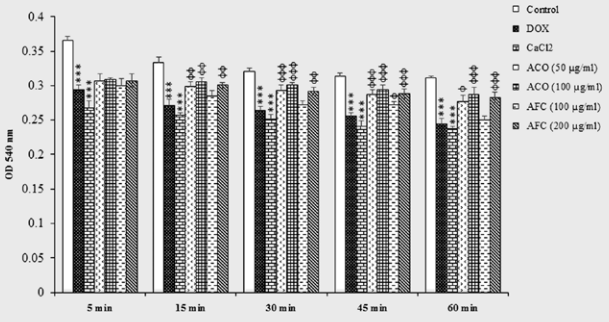
Diverse mechanisms are involved in DOX-induced cardiotoxicity, but it has been suggested that mitochondria have a crucial role in the DOX cardiac toxicity mechanisms. DOX has a high affinity for mitochondrial membrane and therefore accumulates inside the mitochondria. Due to its high redox potential, DOX diverts electrons from complex I of the respiratory chain. Consequently, redox cycling of quinone-semiquinone moiety of DOX leads to the production of ROS and oxidative stress which alerts cardiomyocytes-specific gene expression [5, 32]. Therefore, many attempts have been made to reduce the cardiotoxic effects of DOX through ameliorating mitochondrial dysfunction. Results of the current study showed that the mitochondrial dysfunction markers were elevated in isolated heart mitochondria of DOX-induced heart failure rats in comparison with those of normal rats. These results are consistent with previously published results that have demonstrated mitochondrial oxidative stress and dysfunction are the principal mechanisms of DOX cardiotoxic effects [4].

The interaction of excessive ROS and diverse mitochondrial macromolecules causes GSH depletion and lipid peroxidation. GSH has

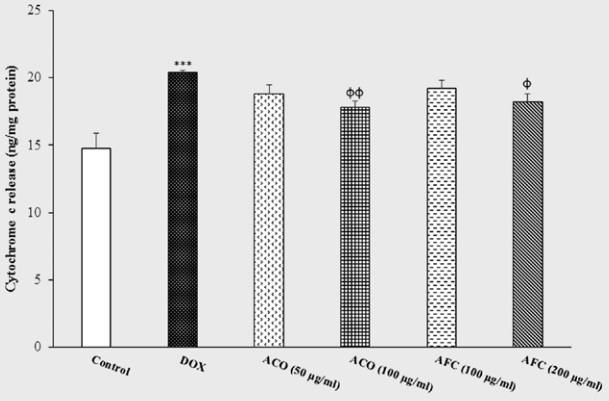
▶ **Table 1** Effect of ACO and AFC on mitochondrial membrane potential collapse (%ΔΨ) in isolated heart mitochondria.

Addition	%ΔΨ				
	Incubation time				
	5 min	15 min	30 min	45 min	60 min
Control	0	9.2 ± 1.7	21.8 ± 1.8	34.4 ± 2.3	39.9 ± 3.1
+ $CaCl_2$	13.1 ± 0.6 * * *	21.8 ± 1.7 * * *	56.7 ± 1.7 * * *	73.4 ± 2.5 * * *	80.7 ± 3.3 * * *
DOX	8.3 ± 0.9 * * *	18.2 ± 1.5 *	32.1 ± 1.9 * * *	44.4 ± 2.6 * *	58.9 ± 3.5 * * *
+ ACO (50 µg/ml)	6.9 ± 1.2	14.8 ± 1.3	27.7 ± 1.6	40.7 ± 1.9	54.7 ± 2.7
+ ACO (100 µg/ml)	8.1 ± 1.0	13.4 ± 1.7 Φ	25.06 ± 1.9 Φ	38.3 ± 2.4 Φ	51.5 ± 2.9
+ AFC (100 µg/ml)	7.9 ± 1.0	16.3 ± 1.0	29.8 ± 1.5	42.1 ± 2.6	57.8 ± 1.34
+ AFC (200 µg/ml)	7.5 ± 0.9	14.5 ± 1.2 Φ	26.9 ± 1.4 Φ	40.05 ± 2.8	53.2 ± 3.3

Mitochondrial membrane potential was determined as the difference in mitochondrial uptake of rhodamine 123 between control and treated mitochondria. Our data were shown as the percentage of mitochondrial membrane potential collapse (%ΔΨ) in all treated (test) groups. Values are expressed as the mean ± SD of 3 separate experiments (n = 3). * $P<0.05$; * * $P<0.01$; * * * $P<0.001$ compared with isolated heart mitochondria of normal rats (Control group) in the same time; Φ $P<0.05$ compared with isolated heart mitochondria of DOX-treated rats (DOX group).



▶ **Fig. 5** Effects of ACO and AFC on mitochondrial swelling in isolated heart mitochondria. Mitochondrial swelling was measured as optical density (OD) by determination of absorbance at 540 nm. Values are expressed as the mean ± SD of 3 separate experiments (n = 3). * * * P < 0.001 compared with isolated heart mitochondria of normal rats (Control group) in the same time; Φ P < 0.05; ΦΦ P < 0.01; ΦΦΦ P < 0.001 compared with isolated heart mitochondria of DOX-treated rats (DOX group).



▶ **Fig. 6** Effects of ACO and AFC on cytochrome c release in isolated heart mitochondria. The amount of expelled cytochrome c from mitochondrial fraction was determined using Rat/Mouse cytochrome c ELISA kit following 60 min of incubation. Values are expressed as the mean ± SD of 3 separate experiments (n = 3). * * * p < 0.001 compared with isolated heart mitochondria of normal rats (Control group); Φ P < 0.05; ΦΦ P < 0.01 compared with isolated heart mitochondria of DOX-induced heart failure rats (DOX group).

a strategic role in the maintenance of suitable redox environment of mitochondria through neutralizing hydrogen peroxide, lipid hydroperoxides, electrophiles, and xenobiotics. Therefore, the depletion of mitochondrial GSH accounts a serious threat to the cell which oxidatively modifies different mitochondrial macromolecules such as lipid, proteins, and DNA. Lipid peroxidation of biomambranes happens in the mitochondria as a result of GSH depletion and oxidative stress. This toxic event inactivates different mitochondrial components such as respiratory chain enzymes and causes mitochondrial dysfunction. Succinate dehydrogenase or respiratory complex II is one of these enzymes that its activity decreases in oxidative stress and the subsequent toxic events. It plays unique role in mitochondrial function and is the only enzyme that contributes in both the citric acid cycle and the electron transport chain. Thus, any impairment of complex II leads to ROS production and the potentiation of oxidative stress [33]. In the present study, the protective effects of ACO and AFC on cardiomyocyte mitochondrial oxidative stress in a rat model of DOX-induced cardiotoxicity were also investigated. The protective effect of different antioxidants on DOX-induced cardiac injury supports the essential role of oxidative stress in the toxicity [6, 34]. Results of this study indicated that ACO and AFC ameliorated mitochondrial oxidative stress by preventing ROS production and membrane lipid peroxidation as well as elevating GSH content and succinate dehydrogenase activity in the isolated heart mitochondria of rats receiving DOX.

Elevated ROS in DOX cardiotoxicity can also attack the thiol cross-linking of the MPT pore region and increase permeability. The opening of MPT pores can lead to MMP collapse (ΔΨm) and mitochondrial swelling. The current results showed that ACO and AFC effectively prevented MMP decrease (%ΔΨm) and mitochondrial swelling as final markers of mitochondrial dysfunction. Release of cytochrome c from mitochondria into cytosol following MPT pore opening is a crucial initiating phase in both apoptotic and necrotic cell death processes in intact cells which are responsible for cardiac damage [5]. In this study, ACO and AFC prevented the release of cytochrome c from the isolated heart mitochondria of rats which leads to apoptosis or necrosis pending on the cellular ATP level. However, ACO was much more effective than AFC at ameliorating the markers of mitochondrial dysfunction in isolated heart mitochondria of DOX-treated rats simply because the effective concentration of ACO was 100 µg/ml compared with the 200 µg/ml concentration of AFC.

Quince and fig have been used in Iranian traditional medicine for the prevention and treatment of cardiovascular diseases [9]. Quince (Cydonia oblonga Mill.) contains polyphenols, organic acids, and free amino acids [35–37]. Fig (Ficus carica L.) possesses antioxidant activity and is a source of phenolic and flavonoid compounds, alkaloids, terpenoids, and saponins [38, 39]. Results of the current study suggest that the bioactive compounds from quince and fig would be valuable for their potential use in the prevention and treatment of heart diseases. It can be also suggested that the cardioprotective effects of quince and fig against DOX-induced cardiotoxicity are partly mediated by their antioxidant effects. Antioxidant supplements improve the benefits of treatment in cancer patients and reduce certain forms of toxicity associated with chemotherapy [40]. However, the authors believe that further *in vivo* and *in vitro* studies are required to clarify what active constituents are responsible for the cardioprotective effects of the 2 edible fruits and to fully explain the mechanisms underlying the effects.

Conclusion

Mitochondrial dysfunction has been involved in DOX-induced cardiotoxic effects. The results of the current research showed that ACO and AFC ameliorate the impairment of cardiac mitochondrial function in DOX-treated rats by preventing mitochondrial ROS generation, lipid peroxidation, swelling, membrane potential decrease (%ΔΨm), and cytochrome c release and also by elevating mitochondrial GSH and complex II activity. Therefore, quince and fig show

potentials to be considered as safe and suitable candidates in managing cancer patients receiving DOX.

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Conflict of Interest

The authors declare that they have no competing interests.

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