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The effect of hydroalcoholic extract of *Cydonia oblonga Miller* leaf on doxorubicin-induced cardiac injury in rat

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Abstract

Background and Objective: Doxorubicin is one of the most common drugs for chemotherapy. The complications of doxorubicin are cardiac toxicity due to oxidative stress. Cydonia oblonga Miller leaf (COL) contains flavonoids and phenolic antioxidants. Due to the presence of antioxidant compounds in COL, the aim of this study was to evaluate the effect of hydroalcoholic extract of COL on doxorubicin-induced cardiac injury in rat.

Materials and Methods: In this experimental investigation, 32 male Wistar rats were divided into 4 groups: control, control under treatment of hydroalcoholic extract of COL, doxorubicin and doxorubicin under treatment of hydroalcoholic extract of COL. In treatment groups, 200 mg/kg of hydroalcoholic extract of COL was injected intraperitoneally one hour after the first dose of doxorubicin for 2 weeks and administered daily. For induction of cardiac toxicity, doxorubicin was injected at a dose of 15 mg/kg intraperitoneally. After two weeks of treatment, the rats were anesthetized with diethyl ether and their heart was removed. After tissue homogenate was prepared, oxidative stress markers were measured using specific kits.

Results: The results of this study demonstrated that doxorubicin increases malondialdehyde and reduced glutathione and catalase activity in the cardiac tissue of rats. Two weeks of treatment with hydroalcoholic extract of COL significantly reduced the malondialdehyde level and increased glutathione. The increase in catalase activity was not statistically significant.

Conclusion: According to the results of this study, COL with phenolic and flavonoid compounds and antioxidant activity seems to attenuate lipid peroxidation and oxidative stress in doxorubicin induced cardiac toxicity.

Key words: Doxorubicin, Cydonia oblonga Miller leaf, Cardiac toxicity, Oxidative stress, Rat

1. Introduction

Today, the importance of cancer has attracted the attention of many researches around the world. The disease, which is considered one of the greatest public health problems, is the second leading cause of death worldwide (1). Currently, the common treatments for this disease are surgery, chemotherapy and radiation therapy (2). Various medicines are used to treat or control cancers. Anthracyclines are a group of antibiotics that are used in chemotherapy and have a very broad clinical application (3). Doxorubicin is a category of anthracyclines and an anticancer drug known as adriamycin and is produced by *Streptomyces peucetius* (4). Doxorubicin is one of the most commonly used drugs to treat a variety of malignancies, including hematologic malignancies and other solid tumors (5,6). The most common side effects of doxorubicin include neutropenia, anemia, leukopenia, thrombocytopenia, congestive heart failure, itching, stomatitis, and general symptoms such as weakness, fatigue, etc. (7-9). The cardiac toxicity developed by this drug has limited its use. Acute injury occurs immediately after treatment and may cause temporary arrhythmia, pericarditis, and temporary impairment of left ventricular function (10). The most important mechanism of cardiac toxicity induced by doxorubicin is oxidative stress.

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Doxorubicin, by producing reactive oxygen species (ROS) and lipid peroxidation, damages the mitochondrial membrane, releases cytochrome C and thus induces apoptosis in cardiomyocytes (11-19).

Due to the role of oxidative stress and the weakness of the antioxidant system in doxorubicin-induced heart damage, the use of natural antioxidant therapy has less side effects in preventing the side effects of this drug. The plant is named *Cydonia oblonga Miller* from the Rosacea family as one of the most antioxidant-rich herbs in the north and northwest of Iran (20,21). Different parts of this plant are used in the traditional treatment of cardiovascular, respiratory, digestive and urinary tract disorders (20,22). The leaves of this plant contain flavonoids and phenolic antioxidants. The main combination of the organic acid is the citric acid and quinine acid (23). Recent studies have shown that the Cydonia oblonga leaf (COL) having flavonoids and phenolic acids has anti-diabetic, anti-hyperlipidemia, and antiarrhythmic effects (24). It has also been shown to have anti-cancer (25) and antioxidant properties (26). Although other medicinal herbs that have side effects with toxic effects, no side effects have been reported from different parts of the Cydonia oblonga (21.23). Due to the richness of the antioxidant compounds in COL and the limitation of studies on the effect of natural antioxidants on cardiac damage caused by doxorubicin, the purpose of this study was to investigate the effect of hydroalcoholic extract of COL on doxorubicin induced cardiac toxicity in rats.

2. Materials and Methods

In this experimental investigation, 32 male Wistar rats weighing 180-230 g (provided by Pasteur institute, Tehran) were used. All of the animals were kept at 21-23 degrees Celsius with a diurnal/nocturnal cycle of 12/12 with appropriate ventilation in groups of 4 in each cage. Subject animals had free access to tap water and specially-produced rat food (provided by Khorak Daam Pars). Handling and methodologies regarding this experimentation and animal care and use were followed thoroughly by the guidelines of national institutes of health of America (NIH) (27,28).

Hydroalcoholic extract of COL was prepared by maceration method (provided by Research Institute of Forests and Rangelands, Herbarium Code: E961100)

The rats were randomly divided into 4 groups: control, control under treatment of hydroalcoholic extract of COL, doxorubicin and doxorubicin under treatment of hydroalcoholic extract of COL. In the treated groups, 200 mg/kg of extract was administered intraperitoneally from one hour after injection of doxorubicin for 2 weeks and daily (29). For induction of cardiac toxicity, doxorubicin was injected intraperitoneally at 15 mg/kg (30). After 2 weeks of treatment, the rats were anesthetized with diethyl ether and then without the perfusion, the chest of the animal was opened and the heart completely removed and then tissue homogenate was prepared using a homogenizer. In order to produce homogenate, tissue was first weighed and then 0.9% of normal saline was added in proportion to their weight and homogenized with a homogenizer at 5,000 rpm for 2 minutes. The homogenized solution was centrifuged at 3,000 rpm for 5 minutes. To prevent degradation of enzymes and proteins, all steps were performed at 4°C. The supernatant solution was isolated from the rest of the homogenate and collected. The solution was used to measure the indexes (31,32).

Measuring the level of malondialdehyde was based on the reaction of thiobarbituric acid (TBA), which is carried out at boiling temperature. In this method, malondialdehyde reacts with thiobarbituric acid and produce a pinkish color with a maximum absorption at 532 nm. All samples were placed in boiling water for 80 minutes in order to react. The solutions were centrifuged at 3000 rpm for 5 minutes and their optical absorption at 532 nm in a spectrophotometer was read (31).

For evaluation of protein, Bradford protein assay method was chosen, in which bovine serum albumin is used as its standard and spectrophotometry is performed on the homogenized heart tissue, prepared as mentioned above (31).

Catalase (CAT) activity was measured by spectrophotometric method at 240 nm. In this method, the catalase enzyme present in the sample, by decomposition of hydrogen peroxide, reduces the absorption of this material at 240 nm, and the activity of the enzyme is measured by the absorption difference in a unit time. By definition, a catalase unit is an enzyme that breaks down a micro-molecule of H2O2 over a period of one minute at 25°C.

For evaluation of glutathione (GSH), 0.1 ml of the supernatant was mixed with Tris buffer (pH 8.2) and DTNB in the test tubes. To this mixture, methanol was added. An empty reactor (without samples) and an empty sample (without DTNB) were also prepared in a similar method. The test tubes were shaken every 5 minutes and settled for 30 minutes, then centrifuged (3000 rpm at RT for 15 minutes). Optical absorption at 412 nm was measured. The color obtained is stable for one hour (33).

All data were expressed as Mean ± Standard Error of the Mean (SEM). One-way analysis of variance (ANOVA) test was used for data analysis and Tukey test was used for significant differences. Statistical analysis of the data was performed in the SigmaStat version 3.5 (2006). Significant value for all analyses were appointed at $p<0.05$.

3. Results

Figure 1 shows the results of lipid peroxidation (malondialdehyde levels, MDA) in rats in different groups. This parameter was significantly higher in the Doxorubicin and Doxorubicin+COL groups than in the control group ($p<0.01$ and $p<0.05$, respectively). In addition, this parameter showed a significant decrease in the doxorubicin+COL as compared to the doxorubicin group ($p<0.05$). In the control+COL group, MDA was not significantly different in comparison with control group.

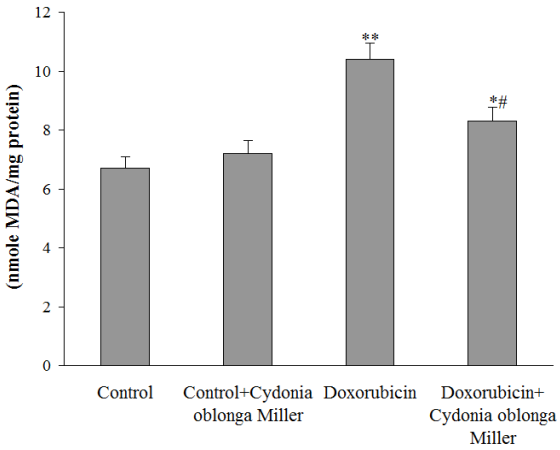

Figure 1. MDA as a lipid peroxidation index in study groups.

* (P<0.05) ** (P<0.01) compared to control group.

(P<0.05) compared to doxorubicin group

Figure 2 shows the results of catalase activity in rats in different groups. This parameter was lower in doxorubicin group than control group, but this difference was not statistically significant ($p>0.05$). In addition, this parameter in the doxorubicin+COL increased as compared to the doxorubicin group, which was not statistically significant ($p>0.05$). In addition, in the control+COL group, no significant difference was found in comparison with control group ($p>0.05$).

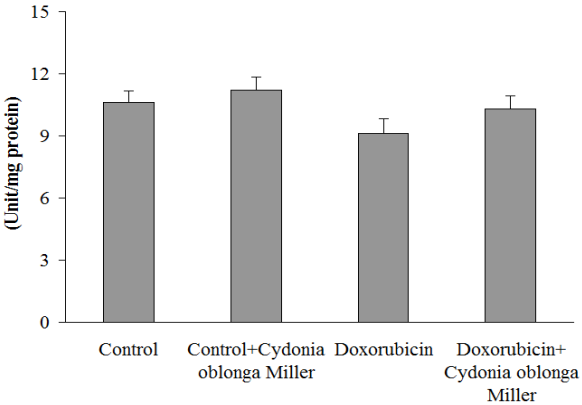

Figure 2. Catalase activity in study groups

Figure 3 shows the results of GSH in rats in different groups. This parameter was significantly lower in doxorubicin group than control group ($p<0.05$). In addition, this parameter showed a significant increase in doxorubicin+COL group as compared to doxorubicin group ($p<0.05$). There was no significant difference between the control group and the control+COL group.

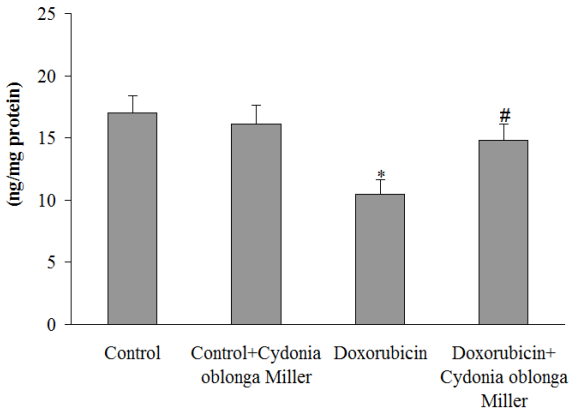

Figure 3. Amount of GSH in study groups.

* (P<0.05) compared to control group.

(P<0.05) compared to doxorubicin group

4. Discussion

The results of this study showed that injection of doxorubicin as an experimental model of cardiac toxicity after two weeks resulted in a significant increase in MDA in the two groups receiving doxorubicin as compared to the control group. In addition, with regard to the activity of catalase and GSH, these two parameters in the groups receiving doxorubicin were lower than the control group, although this difference was not statistically significant. The results in this case indicate that cardiac toxicity is induced by the injection of doxorubicin. The mechanism of this damage can be related to lipid peroxidation and oxidative stress caused by free oxygen radicals, which increases MDA, decreases catalase activity, and reduces glutathione reduction in the rat receiving doxorubicin. In a study by Ghosian Moghadam et al., MDA is considered as an important indicator of lipid peroxidation and oxidative stress (31). In addition, in this regard, the results of Ashrafi et al. in 2014 on tissue damage induced by doxorubicin in rats showed that administration of doxorubicin significantly increases MDA and nitric oxide and decreases the activity of superoxide dismutase and catalase in heart and liver tissues (11). The results of Ashrafi et al. research are consistent with the results of our study.

On the other hand, results of MDA in different groups showed that this parameter in the doxorubicin+COL group shows a significant decrease as compared to doxorubicin group. However, no significant difference was found in the control+COL group as compared to the control group. The activity of catalase in the doxorubicin+COL group as compared to the doxorubicin group showed that this increase was not statistically significant. The amount of GSH in doxorubicin+COL group showed a significant increase as compared to the doxorubicin group, while the control+COL group showed no significant difference as compared to the control group.

Recent studies have shown that the COL have flavonoid and phenolic acid compounds with anti-diabetic, lipid-lowering, anti-cancer and antioxidant effects (24-26). In this regard, in relation to the beneficial effects of COL, Aliasl et al. by reviewing the therapeutic effects of *Cydonia oblonga* on the basis of traditional Iranian medicine and modern herbal medicine, the leaf has various pharmacological effects such as antioxidant, anti-allergic, anti-inflammatory, anti-cancer, antibacterial and anti-colitis effects. It also has beneficial effects on blood pressure and cell blood counts and the treatment of diseases related to the heart, brain and digestive system (20).

In this regard and in relation to the antioxidant effects of this plant, Gholami et al. showed that treatment with the aqueous extract of *Cydonia oblonga* improves the mitochondrial function of myocardium in rat and prevents the formation of ROS, cell membrane lipid peroxidation, mitochondrial degradation, and mitochondrial mucosal collapse. Also, the extract improved oxidative stress by decreasing the activity of glutathione and succinate dehydrogenase, and this effect was attributed to the effect of *Cydonia oblonga* on improving the mitochondrial function and increasing the treatment index of doxorubicin (30). The overall results of this study consistent with our study results. In another study, Hajizadeh Moghadam et al. showed that in the oxidative stress group, the activity of catalase and superoxide dismutase enzymes significantly decreases as compared to the control group, while pre-treatment with different doses of COL significantly increases the activity of these enzymes as compared to the control group, and it was found that treatment with COL increases activity of antioxidant enzymes and can reduce oxidative stress induced by streptozotocin in the cerebral cortex (21). The results of this study are to some extent consistent with the results of our study, although, contrary to the results of Hajizadeh Moghadam et al study, catalase changes were not statistically significant. The probable cause of this difference can be the type of empirical oxidative stress.

Sayed et al. showed that 15 days of treatment with COL in lesion groups induced by 4-nonylphenol improves tissue damage and hormonal levels, and significantly reduces MDA and acetylcholinesterase enzymes, but its the effect was not statistically significant for other oxidative stress enzymes (34). The results of this study on the effect of COL on improving the lipid peroxidation and reduction of MDA matched with our study results. In addition, they did not find significant results in relation to catalase activity, which is similar to the results of our study. Umar et al. examined the anti-oxidant effect of flavonoids derived from leaf and fruit of *Cydonia oblonga* in the hyperlipidemic rats. The results showed that 4 weeks of flavonoid therapy derived from COL significantly improves the lipid profiles. Serum aminotransferases and serum MDA were significantly decreased during this period (29). The results of their study on reducing MDA by the effect of COL flavonoid-derived is similar to our study. According to the results of Umar et al. and our study results, it seems that COL with flavonoid compounds rich in antioxidant properties can act against ROS, lipid peroxidation and oxidative stress. In addition, Abliz and colleagues examined the effects of hydroalcoholic extract of COL in hyperlipidemic rats. COL significantly improved dyslipidemia in rats and decreased MDA and activity of hepatic aminotransferases are inhibited. In addition, the activity of superoxide dismutase and GSH significantly increased (35). The results of their study on the beneficial effects of COL on oxidative stress, especially MDA and a significant increase in GSH, are consistent with our study results.

In addition, the beneficial effects of COL on other cardiovascular diseases have also been studied, so that Zhou et al reported that 8 weeks of treatment with COL had a similar effect on the blood pressure in the rat as compared to captopril as a positive control. In addition, COL in comparison to captopril could reduce the viscosity of the blood and improve erythrocyte deformity (36). The beneficial effects of this extract on the cardiovascular system are consistent with our study results.

Conclusion

The results of this study showed that doxorubicin increases MDA and decreases GSH and catalase activity in the heart tissue of rats. Two weeks of treatment with COL improved lipid peroxidation and oxidative stress. According to the results of this study and the results of recent studies, it seems that COL has beneficial effects due to phenolic and flavonoid compounds and antioxidant activity.

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Conflict of interest

The authors declared no conflict of interest.

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