Investigating the Protective Effects of Quercetin Against Doxorubicin Cardiotoxicity in Rats

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ABSTRACT

Doxorubicin is an anthracycline and is an effective anti-cancer drug. Due to acute and chronic side effects of this drug, including cardiotoxicity, its clinical use is limited. The present study aimed to investigate the protective effect of quercetin on cardiotoxicity caused by doxorubicin. In this study, Wistar rats were divided into 5 groups. In one control group, rats received saline solution (1 mL/kg), and in the other control group (containing quercetin) DMSO solution (1 mL/kg) by gavage for 14 days. In the quercetin group, animals received quercetin (20 mg/kg) by gavage for 14 days. In the doxorubicin group, animals received doxorubicin (25 mg/kg) intraperitoneal for 3 days. In the pretreatment group with quercetin, rats received quercetin (20 mg/kg) by gavage and doxorubicin (25 mg/kg) intraperitoneal for 14 days. After determining the weight, the mice were killed and the heart tissue sample was prepared for analysis. Based on the results obtained from this study, significant weight loss was reported in animals receiving doxorubicin ($P < 0.05$). However, pretreatment with quercetin prevented the weight loss of animals in the doxorubicin group. Pathological examination of heart tissue showed that quercetin has a protective effect against heart damage caused by doxorubicin. In general, it can be concluded from the results of this study that pretreatment with quercetin can prevent the occurrence of cardiotoxicity caused by doxorubicin, which probably caused cardiac protection through its antioxidant properties.

Key Words: *Antioxidant properties, Quercetin, Cardiotoxicity, Doxorubicin*

eIJPPR 2024; 14(4):23-28

HOW TO CITE THIS ARTICLE: Dulf PL, Coadă CA, Florea A, Moldovan R, Baldea I, Dulf DV, et al. Investigating the Protective Effects of Quercetin Against Doxorubicin Cardiotoxicity in Rats. Int J Pharm Phytopharmacol Res. 2024;14(4):23-8. https://doi.org/10.51847/t30RsFhgHG

INTRODUCTION

Today, cancer is considered one of the main causes of death in economically developed countries, and the second major cause of death in developing countries [1, 2]. According to the latest epidemiological studies, cancer incidents are considered one of the main causes of death and several people die from cancer every day [3-5]. According to published reports, the incidence of cancer increases with age. Due to the increasing age of the world population, it is predicted that by 2030, about 70% of malignancies will occur in the age group over 65 years old [4-6]. Common treatments for cancer include surgery, chemotherapy, and radiation. In the long-term administration of anticancer drugs, systemic toxicities are among the limiting factors in treatment. In addition to

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Received: 03 May 2024; **Revised:** 07 August 2024; **Accepted:** 10 August 2024

severe side effects, currently available treatments have resulted in poor survival rates and limited clinical outcomes for many cancers [7].

Doxorubicin (DOX) is an antibiotic with a wide range of antitumor and anticancer (antineoplastic) activity and has been widely used in cancer treatment since the late 60s [8, 9]. Unfortunately, due to damage to non-target tissues and the need to limit the therapeutic doses of doxorubicin and reduce the quality of life of patients during and after treatment, this often complicates cancer treatment. Several mechanisms have been reported for the cardiotoxic and hepatotoxic side effects of doxorubicin, including the production of free radicals, mitochondrial damage, and cytotoxicity [10-13]. It is believed that the production of reactive oxygen species (ROS) and stimulation of cell death (apoptosis) play a central role in the process of toxicity of different tissues such as the heart and liver [13-15]. Cells generally protect themselves from damage caused by free radicals using the antioxidant defense system and moderate the toxicity caused by doxorubicin through the glutathione-dependent antioxidant defense system [16, 17]. During the last decade, significant efforts have been made by medical researchers to develop strategies to prevent cardiac and liver toxicity [18-20]. Among other things, in a study, the protective effect of morphine on cardiotoxicity caused by DOX has been reported [18]. Other researchers have also shown the protective effect of tetradrine 2 and amifostine 3 against damage and cardiac toxicity caused by doxorubicin [19, 20]. Since natural compounds have strong antioxidant effects, special attention is paid to their use to reduce side effects and drug toxicity.

Quercetin is a flavonoid with the scientific name of fenta hydroxyflavone and the formula $C_{15}H_{10}O_7$ is found in plants and foods such as onions, green tea, red wine, and broccoli [21, 22]. Quercetin is a very strong antioxidant and many studies indicate that this flavonoid has many effects including neuroprotective, anti-cancer, anti-inflammatory, and anti-diabetic. Some studies have also shown that quercetin has a protective effect on hepatotoxicity caused by ethanol, acetaminophen, and pesticides, but the exact mechanism of this protective effect is still not fully understood. The importance and necessity of the present study is that cardiotoxicity caused by doxorubicin can be a serious threat to the patient and endanger the life of the person, and in this field, no completely confirmed prevention and treatment method has been found yet. Therefore, investigating new methods such as the use of antioxidants such as quercetin seems necessary in this field. Recently, the use of bioactive foods to prevent various diseases has been considered, and one of the most studied antioxidants is the flavonoid quercetin [23-25].

The present study aimed to investigate the protective effect of the flavonoid quercetin on doxorubicin-induced cardiotoxicity in rats.

MATERIALS AND METHODS

In this study, Wistar rats weighing 200-220 grams were used. The temperature and humidity of the room were kept at the standard level and the animals were exposed to sufficient lighting in the form of 12 hours of light/dark. After the period of acclimatization to the environment, relevant experiments were performed on mice for one week. During the experiments, the ethical principles of working with laboratory animals were followed according to the guidelines.

In this study, Wistar rats were randomly divided into 5 groups (6 rats in each group): the control group of rats received 1mL/kg saline by gavage for 14 days. In the other control group (containing quercetin), rats received DNSO by gavage at a dose of 1mL/kg for 14 days. In the third group (quercetin group), rats received quercetin drug at a dose of 20 mg/kg by gavage for 14 days. In group 4 (doxorubicin), rats received doxorubicin drug intraperitoneally (25 mg/kg) for 3 days on days (12, 13 and 14). Group 5 was related to pretreatment with quercetin. During the 14 days, the rats received the drug quercetin at a dose of 20 mg/kg by gavage, and on days 12, 13, and 14, they received the drug doxorubicin at a dose of 25 mg/kg intraperitoneally. On the 15th day, all mice were killed, their hearts were removed and washed with normal saline solution, and the samples were transferred to 10% formalin and then to 4% formalin solution for one week.

The heart sample was sent in 4% formalin solution for cutting and preparation of pathology slide using common methods of tissue passage and preparation of pathological sections. Using a microtome, slices with a thickness of 5 microns were prepared and prepared by the hematoxylin-eosin staining method [26]. Examination and observations of pathological sections were done by a pathologist.

RESULTS AND DISCUSSION

The results of changes in the weight of rats in the studied groups are presented in **Figure 1**. The results of the studies showed that the changes in animal weight in the doxorubicin group were significantly different compared to the control group ($P<0.05$). However, no significant difference was observed between the treatment and control groups.

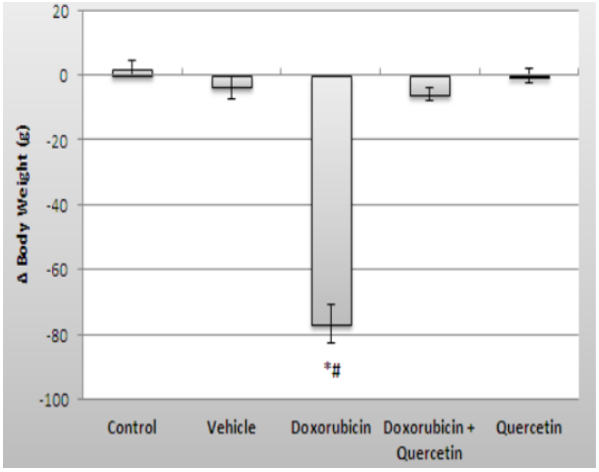

Figure 1. The results of changes in the weight of rats in the studied groups (Mean ± SD). $P<0.05^{*}$ compared to the control group, $P<0.05^{\#}$ compared to the doxorubicin + quercetin group.

Histopathological changes of all groups are compared in **Figures 2-5**. **Figure 2** shows a view of the heart in the group receiving DMSO. In the group treated with DMSO, cardiac cells and their cellular structure are normal, and very few blood vessels are observed. Myocardial fibers and muscle fibers are normal.

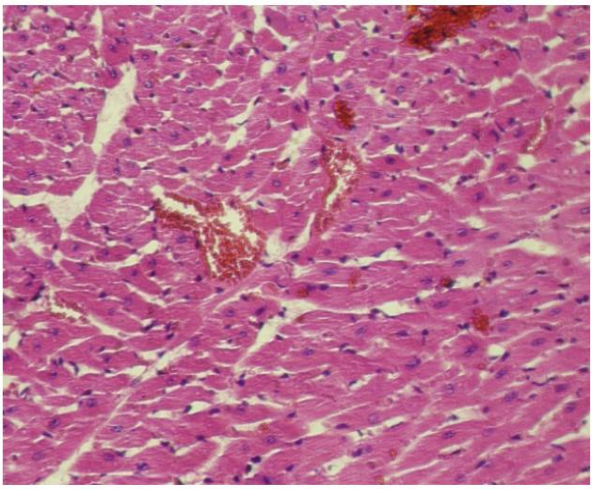

Figure 2. A view of the heart in the group receiving DMSO.

Figure 3 shows a view of the heart in the group receiving quercetin. In this group, cardiac muscle fibers and cardiac cells are normal, only a very small amount of striated state is not clear.

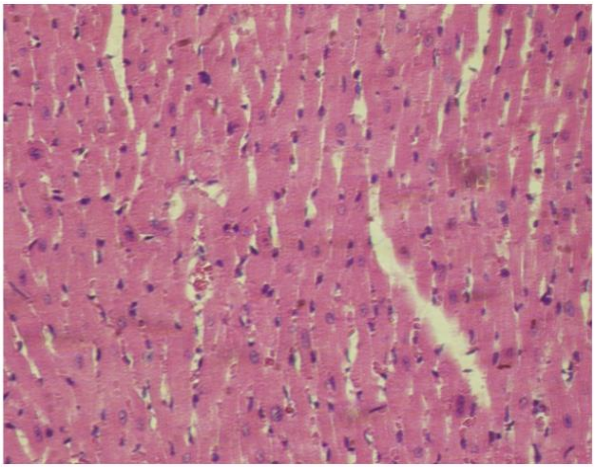

Figure 3. A view of the heart in the group receiving quercetin.

Figure 4 shows a view of the heart in the group receiving doxorubicin. The vascular effects of the drug are well known and the observation of hyperemia is very clear. Loss of muscle fibers and lack of clarity of muscle fibers as well as collagen fibers are observed.

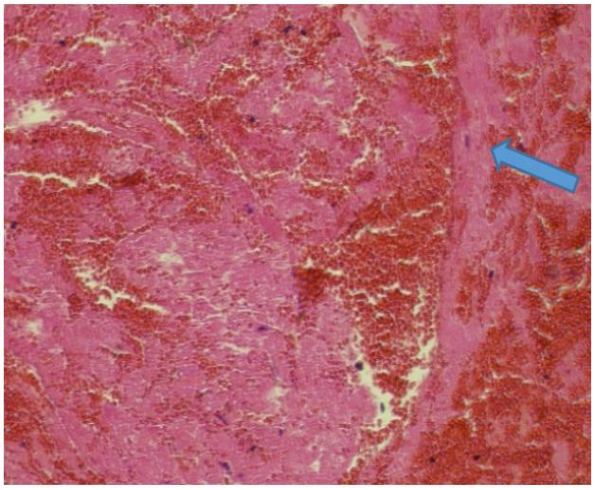

Figure 4. A view of the heart in the group receiving doxorubicin.

Figure 5 shows a view of the heart in the group receiving doxorubicin plus quercetin. Compared to the doxorubicin group, the loss of muscle fibers is observed in a much lower amount, which shows the therapeutic effect of quercetin, and collagen fibers are also observed in a very small amount.

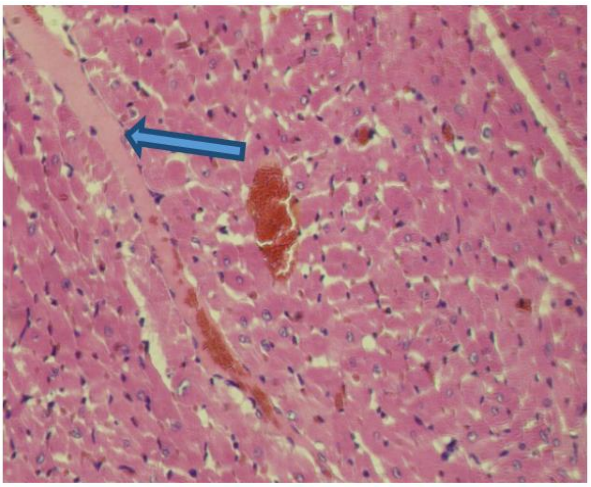

Figure 5. A view of the heart in the group receiving doxorubicin plus quercetin.

Doxorubicin is an anthracycline anticancer drug that is used in the chemotherapy of a wide range of cancers. Due to side effects, including cardiotoxicity, renal and hepatic toxicity, and the use of this drug is limited [10, 11]. This study aimed to investigate the protective effect of quercetin on doxorubicin-induced cardiotoxicity in male rats. In the study of changes in the body weight of mice, it was well established that pretreatment with quercetin prevented the severe loss of body weight in animals receiving doxorubicin. The researchers propose the purpose of measuring the body weight of mice to obtain a general indicator of the health state of mice following the administration of chemicals.

The findings of these studies generally show the same results in the effect of toxins on body weight, which is associated with a serious decrease. An increase in oxidative and metabolic stress Disturbance due to the presence of free radicals is proposed as the main reason for reducing the natural rate of body weight changes following the administration of various toxic substances [27].

Based on the histological findings of the present study, the administration of quercetin was able to provide adequate cardiac protection against the toxicity caused by doxorubicin. Doxorubicin at a dose of 25 mg/kg for 3 days caused cardiac tissue damage, compared to the control group, and pretreatment with quercetin (for two weeks) could prevent the cardiotoxicity caused by doxorubicin. By administering quercetin together with doxorubicin, compared to the doxorubicin group, the state of muscle fibers is much less, as well as collagen fibers are observed in a very small amount, which shows the pre-treatment effect of quercetin. In this regard, our studies on the effect of quercetin on liver protection showed that pretreatment with quercetin could reduce the clear increase in the level of liver enzymes ALT, AST, ALP, and the amount of malondialdehyde (MDA, a biomarker of oxidative stress) caused by doxorubicin toxicity. In addition, quercetin was able to increase the amount of SOD in the quercetin-doxorubicin group. The above beneficial effects can be related to the antioxidant effects and reduction of oxidative stress of this flavonoid. In other words, quercetin stabilizes cell membranes by preventing lipid peroxidation. The role of oxidative stress and the intervention of reactive oxygen species in the pathogenesis of doxorubicin cardiotoxicity has been proven [28].

The protective effect of quercetin on ethanol hepatotoxicity has been reported, and the inhibitory role of the flavonoid quercetin on oxidative stress has been pointed out [29]. In the investigation of the protective effect of quercetin extracted from black tea extract on lipid peroxidation and the changes of antioxidant enzymes caused by the toxicity of insecticides on rat liver, it was reported that the aqueous extract of black tea significantly reduces the level of fat peroxidation and also, the level of antioxidant factors such as SOD (superoxide dismutase) and GP_x (glutathione peroxidase) to the same level as the control group, and these changes are reported to be due to the antioxidant effects of black tea polyphenols [30].

In another study, the protective effect of quercetin as an antioxidant in ethanol-induced acute gastric ulcers was investigated. An increase in the level of MDA as well as a decrease in the amount of SOD and GP were observed significantly compared to the control group. These researchers also expressed the protective effect of quercetin on gastric ulcers caused by ethanol due to the antioxidant activity of quercetin [31]. Also, quercetin has shown a protective effect in acute hepatotoxicity caused by carbon tetrachloride. Administering quercetin in the toxicity model by gavage for 2 weeks caused a decrease in the MDA level and a decrease in the increased level of liver enzymes compared to the group poisoned with carbon tetrachloride. Also, the examination of SOD and GP_x enzymes showed that the level of these enzymes in the group poisoned with carbon tetrachloride decreased significantly compared to the control group, but in the group treated with quercetin, it increased significantly compared to the poisoned group [32]. In another study that looked at the protective effect of quercetin and curcumin in liver and kidney toxicity induced by paracetamol, quercetin was able to limit liver damage well [30-32].

According to the aforementioned combination, probably quercetin protects the heart against the oxidative effects of doxorubicin toxicity through its antioxidant properties. Therefore, after conducting randomized controlled trials and obtaining positive results, this flavonoid can be recommended as a drug to prevent oxidative liver damage caused by doxorubicin in cancer patients and as an accessible source with antioxidant properties so it can be used as a food supplement or through the pharmaceutical industry at the same time as doxorubicin. Whether quercetin reduces the therapeutic effects of doxorubicin or

not remains unclear in this study, and the possibility of comparison in terms of effects in cases with neoplasia has not been provided, and future and wider studies are needed. In this study, induction of toxicity with doxorubicin was performed in healthy mice. It is suggested that this study be done in a cancer model as well. In addition, the change in the level of cardiac enzymes and malondialdehyde should be investigated. In the present study, doxorubicin was administered intraperitoneally, it is suggested to use it intravenously in the next studies and use different doses of quercetin.

CONCLUSION

The present study aimed to investigate the protective effect of quercetin on cardiotoxicity caused by doxorubicin. Based on the results obtained from this study, significant weight loss was reported in animals receiving doxorubicin. However, pretreatment with quercetin prevented the weight loss of animals in the doxorubicin group. Pathological examination of heart tissue showed that quercetin has a protective effect against heart damage caused by doxorubicin. In general, it can be concluded from the results of this study that pretreatment with quercetin can prevent the occurrence of cardiotoxicity caused by doxorubicin, which probably caused cardiac protection through its antioxidant properties.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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