IJKD KIDNEY DISEASES

Protective Effect of Quercetin Against Oxidative Stress-induced Toxicity Associated With Doxorubicin and Cyclophosphamide in Rat Kidney and Liver Tissue

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Original Paper

Introduction. Doxorubicin and cyclophosphamide are widely used anticancer drugs with substantial toxicity in noncancerous tissue resulting from oxidative damage. Quercetin is a potent antioxidant compound. We hypothesized that quercetin administration would ameliorate the toxic effects of doxorubicin and cyclophosphamide prior to pregnancy.

Materials and Methods. Cyclophosphamide, 27 mg/kg, and doxorubicin, 1.8 mg/kg, were administered to rats as intraperitoneal doses once every 3 weeks for a total of 10 weeks with or without concurrent treatment with quercetin, 10 mg/kg/d. Oxidative stress parameters were evaluated in maternal kidney and liver tissues after gestation.

Results. Doxorubicin was associated with elevated kidney tissue malondialdehyde relative to the controls and quercetin only treatment ($P < .05$). Both cyclophosphamide and doxorubicin were associated with elevated malondialdehyde levels in the liver tissue ($P < .05$). Doxorubicin treatment was associated with decreased liver glutathione peroxidase ($P < .05$). Quercetin treatment suppressed the accumulation of malondialdehyde and increased glutathione peroxidase levels during doxorubicin and cyclophosphamide treatment ($P < .05$)

Conclusions. Treatment with quercetin in patients receiving doxorubicin and cyclophosphamide results in therapeutic restoration of homeostatic expression of the antioxidant parameters, reducing oxidative damage to the liver and kidney.

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INTRODUCTION

The cytotoxic drugs used in chemotherapy are targeted at rapidly dividing cells and commonly exert toxic effects on both tumor cells and healthy tissues with rapidly proliferating cells.[1] Doxorubicin intercalates within the DNA of rapidly dividing tumor cells, blocking cell cycle progression in the G2 phase.[2,3] Clinical use of doxorubicin has been constrained due to multi-organ toxicity that includes damage to the liver and kidneys.[4,5] Nicotinamide adenine dinucleotide phosphate reductases catalyze the formation of doxorubicin semiquinone free radical, which, in the presence of oxygen, generates superoxide free radicals.[6] The administration of doxorubicin to women prior to pregnancy is reportedly associated with renal apoptosis. The generation of superoxide radicals following doxorubicin exposure results in excessive apoptosis.[7,8]

Cyclophosphamide is a common immunosuppressant cancer drug. Therapeutic doses of cyclophosphamide are limited by the onset of liver

and kidney toxicity.[9] Oxidative stress, including increased lipid peroxidation (malondialdehyde) and glutathione depletion, have been associated with cyclophosphamide toxicity. The pathology of cyclophosphamide toxicity includes a significant reduction in smooth endoplasmic reticulum, increased autophagocytosis and sequestration of glycogen, and progressive loss of structural density in the mitochondria.[10-13] Cell structure and function is fundamentally disrupted under conditions of oxidative stress and can be detected in changes in the carbohydrate, lipid, and DNA profile of the affected tissues.[14,15] Oxidative stress results from the imbalance of antioxidants and free radicals. An array of enzymes ameliorate the impact of free radicals under normal physiologic conditions, including superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, resulting in the protection of cellular membranes and organelles.[16]

Antioxidant defenses generally protect tissues from the harmful effects of oxidative stress and recent preclinical studies suggest that the antioxidant properties of quercetin can enhance protection from oxidative damage in the heart, brain, and other tissues during ischemic reperfusion or following exposure to free radical-generating compounds.[17-20] In response to these findings, we hypothesized that concurrent treatment with quercetin would reduce liver and kidney toxicity during exposure to doxorubicin and cyclophosphamide.

MATERIALS AND METHODS

Experimental Treatments

Experimental chemotherapy treatment with cyclophosphamide (Molekula, Shaftesbury, Dorset, UK) and doxorubicin (Sigma-Aldrich, Toronto, Canada) was applied in rodent model system. Typical clinical doses were used to determine the treatment dosages in the experimental animals based on the surface area to weight ratio. Single intraperitoneal doses of cyclophosphamide (27 mg/kg) and doxorubicin (1.8 mg/kg) were administered once every 3 weeks over a course of 10 weeks for a total of 4 doses.[21]

Quercetin dehydrate 97% suspended in corn oil (Alfa Aesar, Germany) was administered by oral gavage at a dose of 10 mg/kg/d.[22] Control animals received corn oil alone.

Animals

A total of 36 female Wistar rats weighing approximately 250 g each were used in this study. Two days after drug treatment, the female rats were co-housed with a male rat for breeding, with 4 females and 1 male in each breeding cage. Pregnancy was determined by the presence or absence of a vaginal plug. The day of plug release was considered gestation day 1, and male rats were removed from the cage following confirmation of pregnancy. No restriction of food or water was applied. All animals were exposed to a 12-hour alternate schedule of light and darkness (lights on at 06:00 AM), and the ambient temperature was maintained at 22 ± 2°C at all times.

The local ethics committee of the Medical Sciences Experimental Research (2012/A-28) and Application Center (MSESAC) at Inonu University reviewed and approved all experimental procedures. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Experimental Groups

A total of 6 experimental groups consisting of 6 randomly selected rats each were established: control, quercetin, cyclophosphamide, doxorubicin, cyclophosphamide plus quercetin, and doxorubicin plus quercetin. Maternal kidney and liver tissues were removed for evaluation of antioxidant parameters following gestation, including catalase activity, malondialdehyde antioxidant, superoxide dismutase (SOD), glutathione, and glutathione peroxidase activity.

Biochemical Analysis

Ice-cold Tris–HCl buffer (0.1 M, pH 7.5), supplemented with phenylmethylsulfonyl fluoride (1 mM) and protease inhibitor, was used to suspend the liver and kidney tissues following digestion using a tissue homogenizer (IKA ultra turrax T 25 basic) at 16 000 rpm for 2 minutes at 4°C. Tissue homogenates were used in all biochemical analyses.

Malondialdehyde and Glutathione

The addition of thiobarbituric acid to tissue homogenates and the measurement of light absorbance at 535 nm and 520 nm in a spectrophotometer was used to measure thiobarbituric acid reactive substances including malondialdehyde were measured by the addition

of thiobarbituric acid to tissue homogenates and the measurement of light absorbance at 535 nm and 520 nm in a spectrophotometer as previously described; the results were reported as nmol/g wet tissue.[23] The spectrophotometric Ellman method was used to measure glutathione in kidney and liver homogenate by the reduced glutathione assay, with the results reported as nmol/g wet tissue.[24]

Superoxide Dismutase Assay

The total reduction of nitro blue tetrazolium by the superoxide anion produced by xanthine and xanthine oxidase was used to quantify SOD activity.[25] The quantity of protein required to inhibit nitro blue tetrazolium reduction by 50% was defined as 1 unit of SOD activity, with the results reported as U/mg protein. The Lowry method was used to determine the protein content of tissue homogenate samples.[26]

Determination of Catalase Activity

The Aebi method was used to quantify catalase activity as the rate constant k (dimension, s−1,k) of hydrogen peroxide (initial concentration, 10 mM) as indicated by absorbance at 240 nm in a spectrophotometer, with activity reported as k (constant rate) per g protein (U/g). [4]

Determination of Glutathione Peroxidase Activity

The Paglia and Valentine method of measuring oxidation of reduced nicotinamide adenine dinucleotide phosphate in a spectrophotometer at 340 nm was used to measure glutathione peroxidase activity, which was reported as U/g protein.[27] The Lowry method was used to determine the protein content of tissue homogenate samples.[26]

Statistical Analysis

The Microsoft Excel and the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA) were used to conduct all statistical analyses. Multiple analyses of variance, followed by the post hoc protected Tukey test was used to make comparisons among multiple groups. Parameters within groups were analyzed using the Student *t* test, with correction for multiple comparisons. A *P* value less than .05 was considered significant. The results were expressed as mean ± standard error of mean.

RESULTS

Effect of Quercetin on Malondialdehyde Levels

Doxorubicin treatment was associated with elevated malondialdehyde in the renal and liver tissues relative to the control or quercetin treatment groups ($P < .05$, Figure 1). Quercetin treatment ameliorated the induction of malondialdehyde in the kidney in the group treated with doxorubicin or cyclophosphamide plus quercetin relative to the doxorubicin group ($P < .05$, Figure 1). Quercetin also suppressed malondialdehyde levels of the liver tissue in the doxorubicin and cyclophosphamide groups; the malondialdehyde level was lower in the quercetin group relative to the control group ($P < .05$, Figure 1). Malondialdehyde levels in the kidney tissues were lower than in the liver tissues of the controls, cyclophosphamide, and cyclophosphamide plus quercetin groups ($P < .05$; Figure 1).

Effect of Quercetin on Qlutathione Levels

Quercetin treatment resulted in elevated glutathione levels that did not meet the statistically significance level (Figure 2). Cyclophosphamide and doxorubicin treatments were associated with decreased glutathione levels; however, the decrease was not significant compared to the controls (Figure 2). Glutathione levels in the cyclophosphamide plus quercetin and doxorubicin plus quercetin groups were higher than in the

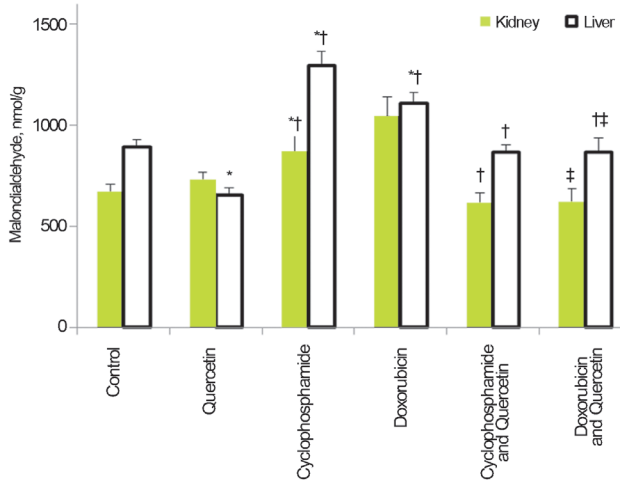

Figure 1. The effect of quercetin on malondialdehyde levels in kidney and liver tissues in rats with doxorubicin- and cyclophosphamide-induced oxidative stress. Data are expressed as mean ± standard error of mean.
*$P < .05$ compared to the control group
†$P < .05$ compared to the quercetin group
‡$P < .05$ compared to the doxorubicin group

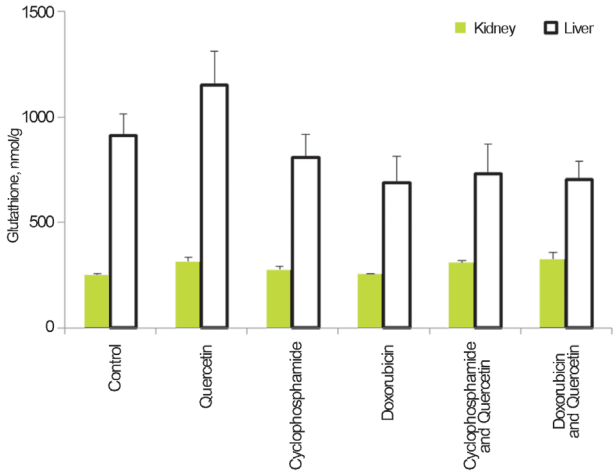

Figure 2. The effect of quercetin on glutathione levels in kidney and liver tissues in rats with doxorubicin- and cyclophosphamide-induced oxidative stress. Data are expressed as mean ± standard error of mean.

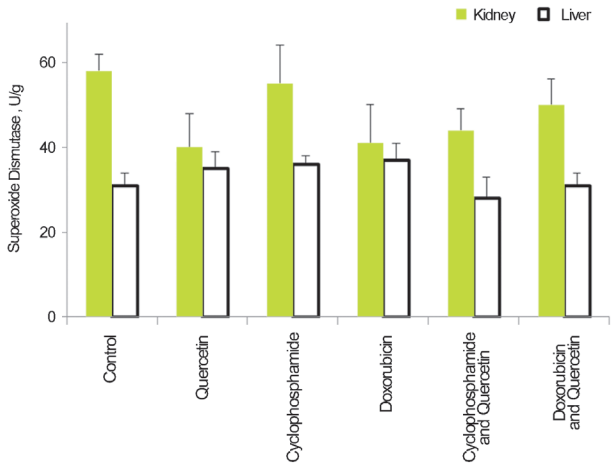

Figure 3. The effect of quercetin on superoxide dismutase levels in kidney and liver tissues in rats with doxorubicin- and cyclophosphamide-induced oxidative stress. Data are expressed as mean ± standard error of mean.

control group in the kidney tissues, but these differences were not significant (Figure 2). Analysis of glutathione in the kidney and liver tissues in each group revealed that glutathione levels in the kidney tissue were lower than in the liver tissue among the control, cyclophosphamide, doxorubicin, and cyclophosphamide plus quercetin groups ($P < .05$; Figure 2). However, glutathione levels in the quercetin group were higher in the kidney tissue than in the liver tissue ($P < .05$; Figure 2).

Effect of Quercetin on Superoxide Dismutase

There were no significant differences in the SOD levels of the kidney and liver tissues among the experimental and control groups (Figure 3). Superoxide Dismutase levels were elevated in the kidney tissue relative to the liver tissue in the control group, but not in the other experimental groups ($P < .05$; Figure 3).

Effect of Quercetin on Catalase Activity

There was no significant difference in catalase levels in the kidney and liver tissues among the six experimental groups (Figure 4). Analysis of catalase levels in each group in the kidney and liver tissues indicated that catalase levels in the kidney tissue were lower than in the liver tissue in the control and cyclophosphamide plus quercetin groups ($P < .05$; Figure 4).

Effect of Quercetin on Glutathione Peroxidase

No significant differences were observed

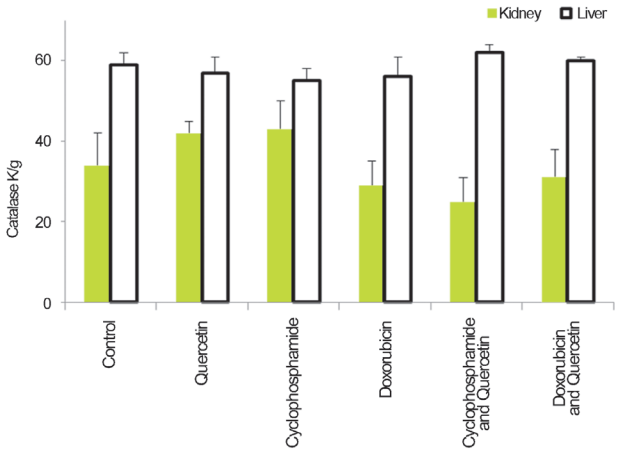

Figure 4. The effect of quercetin on catalase levels in kidney and liver tissues in rats with doxorubicin- and cyclophosphamide-induced oxidative stress. Data are expressed as mean ± standard error of mean.

between the experimental groups in liver or kidney glutathione peroxidase levels (Figure 5). Although doxorubicin and cyclophosphamide treatments were associated with decreased glutathione peroxidase in the liver tissue versus the controls ($P < .05$, Figure 5), there was no significant differences in the total glutathione peroxidase activity (Figure 5). Only the quercetin group exhibited increased glutathione peroxidase levels relative to the doxorubicin plus quercetin and the cyclophosphamide plus quercetin groups in the liver tissue ($P < .05$, Figure 5). Glutathione peroxidase levels in the doxorubicin group were elevated as compared with the doxorubicin plus

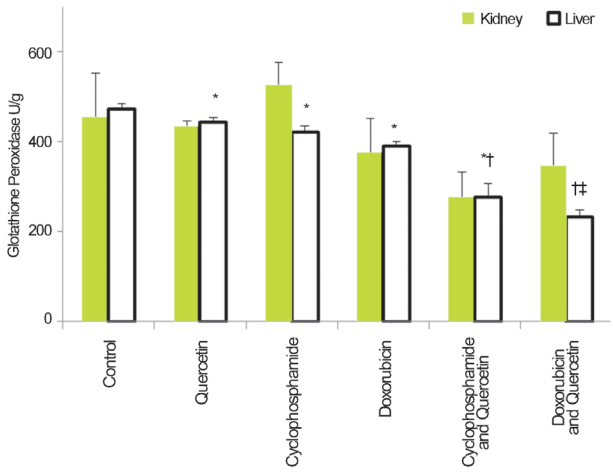

Figure 5. The effect of quercetin on glutathione peroxidase levels in kidney and liver tissues in rats with doxorubicin- and cyclophosphamide-induced oxidative stress. Data are expressed as mean ± standard error of mean.
*$P < .05$ compared to the control group
†$P < .05$ compared to the quercetin group
‡$P < .05$ compared to the doxorubicin group

quercetin concurrent treatment ($P < .05$, Figure 5). The decrease in glutathione peroxidase associated with cyclophosphamide treatment was partially reversed by quercetin concurrent treatment ($P < .05$, Figure 5). Across all the groups, there was no significant differences in glutathione peroxidase levels between the kidney and liver tissues.

DISCUSSION

Elevated malondialdehyde and decreased glutathione peroxidase following doxorubicin exposure results in increased oxidative stress of the hepatic tissues. Quercetin treatment enhanced protection from oxidative stress by suppressing malondialdehyde and elevating glutathione peroxidase expression in the liver. The impact of doxorubicin toxicity was greater in the liver tissue relative to the kidney, likely as a result of the concentration of doxorubicin in the liver during the detoxification process.

Similar to the toxic effects observed in doxorubicin-exposed rats, rats treated with cyclophosphamide exhibited elevated malondialdehyde and enhanced oxidative stress in both the liver and kidney tissues. Cyclophosphamide treatment generates free radicals that can directly damage epithelial and endothelial structures. Damage to DNA is another consequence of intracellular reactive oxygen species generation.[28] Lipid peroxidation may also result from reactive oxygen species generation, but there is limited evidence that this process contributes to the antitumor effects of cyclophosphamide.[29] The present work clearly demonstrates the potential effects of cyclophosphamide-induced lipid peroxidation on liver and kidney tissue. Increased accumulation of malondialdehyde can result in cellular degradation and other biochemical changes as well as apoptosis.[30] The production of free radicals such as superoxide anion radical and hydroxyl radical during cyclophosphamide exposure causes oxidative stress. Oxidative stress can include membrane perturbation through the elevation of malondialdehyde and increased lipid peroxidation.[31] Candidate compounds for reducing liver and kidney toxicity during cyclophosphamide exposure include antioxidant substances such as quercetin.

Cyclophosphamide-associated hepatotoxicity limits clinical use as an anticancer chemotherapeutic. The precise mechanism of cyclophosphamide-associated hepatotoxicity remains unclear. Recent studies suggest that imbalanced oxidant and antioxidant generation results in the release of pro-inflammatory cytokines which contribute to hepatic damage during cyclophosphamide exposure.[5,32]

The toxic effects of doxorubicin on the liver and kidney is mediated by oxidative stress, however the mechanism of oxidative stress is not well understood.[33-35] Several hypotheses have been proposed to explain the prevalence of oxidative damage during doxorubicin exposure. One idea is that doxorubicin results in the generation of oxidant radicals by both enzymatic and nonenzymatic pathways.[36,37] During the transposition of doxorubicin to semiquinone form, electrons can be captured by oxygen, producing superoxide radicals. Previous studies have reported elevated malondialdehyde following doxorubicin exposure, suggesting that cellular membranes are primary targets of doxorubicin-mediated oxidative stress damage.[38-42] The present study is consistent with these previous reports. Recent studies have also demonstrated that doxorubicin exposure results in increased total oxidant generation in the liver, kidney, and heart.[33-35,39] Another study has demonstrated that doxorubicin exposure results in overproduction of oxidant radicals, mitochondrial dysfunction and declining respiration and swelling in rats with doxorubicin-induced hepatotoxicity.[43]

Imbalance between oxidant and antioxidant

generation produces oxidative stress. Increased generation of oxidant radicals overwhelms the ability of antioxidants to absorb these damaging compounds, resulting in oxidative stress damage. Oxidant radicals are produced under physiologic conditions within the mitochondria and the peroxisome, where antioxidants limit oxidative stress damage. The enormous production of oxidant radicals during doxorubicin exposure diminishes the available pool of antioxidants. A previous study suggested that doxorubicin exposure was associated with declining antioxidant parameters, including glutathione peroxidase, SOD, and catalase. Furthermore, this study also demonstrated that doxorubicin exposure results in elevated thiobarbituric acid reactants, an important indicator of oxidative damage.[44] Oxidative damage to the liver following doxorubicin-exposure was attributed to elevated oxidants, such as malondialdehyde, and declining antioxidant parameters, such as catalase, SOD, and reduced glutathione in one report.[43] It has been suggested that quercetin can attenuate doxorubicin-associated hepatotoxicity in non-cancerous liver tissue.[44] Many biological functions have been attributed to quercetin, including antioxidant, anti-inflammatory, and anti-hypertrophic activities.[42] A recent study concluded that quercetin has anticancer properties and an overall chemoprotective effect as a result of the interruption of the cell cycle at the G1 phase and/or G2/M phase through elevated expression of p53, p21, and p27 and declining Bcl-xL expression.[46] Quercetin may also have applications in the treatment of heart disease through the reduction of blood pressure and reduced cardiac hypertrophy and proteinuria.[45] Quercetin enhances the antitumor effects of doxorubicin, while simultaneously limiting cytotoxicity in noncancerous tissue, and several proposals have been put forward to explain the mechanism of action of quercetin.[47] Quercetin chelates metal ions such as iron and copper, which are capable of scavenging free radicals. Quercetin posttreatment inhibits angiotensin-converting enzyme activity in doxorubicin-exposed rats, resulting in decreased lipid peroxidation. Quercetin may also inhibit nuclear factor kappa-B, a redox-sensitive transcription factor, reducing expression of pro-inflammatory matrix metalloproteases and elevating nitric oxide levels.[42] Quercetin treatment elevates the nonenzymatic antioxidant capacity of plasma and suppresses lipid peroxidation.[48,49] Quercetin has also been reported to restore mitochondrial function and protect against DNA double strand breaks in H9c2 cells exposed to doxorubicin. Quercetin attenuates oxidative stress through the reduction of nicotinamide adenine dinucleotide phosphate oxidase-mediated superoxide production and promotes lipid beta oxidation.[50]

CONCLUSIONS

The results of the present study demonstrate that liver and kidney cytotoxicity during doxorubicin and cyclophosphamide are mediated by oxidative stress resulting from the elevation of oxidant radicals and the decline in antioxidants such as glutathione peroxidase, SOD, and catalase. Quercetin, a flavonoid, ameliorated the cytotoxic effects of doxorubicin and cyclophosphamide on the liver and kidney through the elevation of antioxidant expression and the suppression of lipid peroxidation. Therefore, quercetin may be a candidate therapeutic for the prevention of toxicity during doxorubicin exposure. Clinical use of quercetin in cancer treatment is significantly limited due to very poor delivery features of the compound. Nevertheless, more research is needed to evaluate the therapeutic effects of quercetin on doxorubicin-mediated hepatotoxicity and renal toxicity.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Van CK, Heyns L, De SF, et al. Cancer during pregnancy: an analysis of 215 patients emphasizing the obstetrical and the neonatal outcomes. J Clin Oncol. 2010;28:683-9.
2. El-Moselhy MA, El-Sheikh AA. Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed Pharmacother. 2014;68:101-10.
3. Attia SM, Bakheet SA. Effect of dihydrokainate on the capacity of repair of DNA damage and apoptosis induced by doxorubicin. Mutagenesis. 2013;28:257-61.
4. Tulubas F, Gurel A, Oran M, Topcu B, Caglar V, Uygur E. The protective effects of omega-3 fatty acids on doxorubicin-induced hepatotoxicity and nephrotoxicity in rats. Toxicol Ind Health. 2015;31:638-44.
5. Ibrahim MA, El-Sheikh AA, Khalaf HM, Abdelrahman AM. Protective effect of peroxisome proliferator activator receptor (PPAR)-alpha and -gamma ligands against methotrexate-induced nephrotoxicity. Immunopharmacol Immunotoxicol. 2014;36:130-7.

6. Rehman MU, Tahir M, Khan AQ, et al. D-limonene suppresses doxorubicin-induced oxidative stress and inflammation via repression of COX-2, iNOS, and NFkappaB in kidneys of Wistar rats. Exp Biol Med (Maywood). 2014;239:465-76.
7. Pedrycz A, Wieczorski M, Czerny K. Late effects of adriamycin single dose on fetal rat kidney-ultrastructural assessment. Environ Toxicol Pharmacol. 2005;20:157-60.
8. Pedrycz A, Czerny K. Immunohistochemical study of proteins linked to apoptosis in rat fetal kidney cells following prepregnancy adriamycin administration in the mother. Acta Histochem. 2008;110:519-23.
9. Raza M, Alghasham AA. Desferrioxamine protects against toxic damage to liver and kidney induced by cyclophosphamide. Int J Health Sci (Qassim). 2011;5:15-7.
10. Draeger J, Peter G, Hohorst HJ. Deactivation of cyclophosphamide (NSC-26271) metabolites by sulfhydryl compounds. Cancer Treat Rep. 1976;60:355-9.
11. Topal T, Oztas Y, Korkmaz A, et al. Melatonin ameliorates bladder damage induced by cyclophosphamide in rats. J Pineal Res. 2005;38:272-7.
12. Manesh C, Kuttan G. Effect of naturally occurring isothiocyanates in the inhibition of cyclophosphamide-induced urotoxicity. Phytomedicine. 2005;12:487-93.
13. Abd-Allah AR, Gado AM, Al-Majed AA, Al-Yahya AA, Al-Shabanah OA. Protective effect of taurine against cyclophosphamide-induced urinary bladder toxicity in rats. Clin Exp Pharmacol Physiol. 2005;32:167-72.
14. Farias JW, Furtado FS, Guimaraes SB, Silva Filho AR, Vasconcelos PR. Oxidative stress parameters in women with breast cancer undergoing neoadjuvant chemotherapy and treated with nutraceutical doses of oral glutamine. Acta Cir Bras. 2011;26 Suppl 1:82-7.
15. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. 3rd ed. New York: Oxford University Press; 1999.
16. Ferreira ALA, Matsubara LS. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. Rev Ass Med Brasil. 1997;43:61-8.
17. Ray G, Batra S, Shukla NK, et al. Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast Cancer Res Treat. 2000;59:163-70.
18. Feng WH, Wei HL, Liu GT. Effect of PYCNOGENOL on the toxicity of heart, bone marrow and immune organs as induced by antitumor drugs. Phytomedicine. 2002;9:414-8.
19. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem. 1995;41:1819-28.
20. Bayne AC, Sohal RS. Effects of superoxide dismutase/catalase mimetics on life span and oxidative stress resistance in the housefly, Musca domestica. Free Radic Biol Med. 2002;32:1229-34.
21. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003;79:829-43.
22. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. J Natl Cancer Inst Monogr. 2005;12-7.
23. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86:271-8.
24. Ilhan N, Halifeoglu I, Ozercan HI, Ilhan N. Tissue malondialdehyde and adenosine triphosphatase level after experimental liver ischaemia-reperfusion damage. Cell Biochem Funct. 2001;19:207-12.
25. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. 1988;34:497-500.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.
27. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158-69.
28. Sulkowska M, Sulkowski S, Skrzydlewska E, Farbiszewski R. Cyclophosphamide-induced generation of reactive oxygen species. Comparison with morphological changes in type II alveolar epithelial cells and lung capillaries. Exp Toxicol Pathol. 1998;50:209-20.
29. Matalon ST, Ornoy A, Lishner M. Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta). Reprod Toxicol. 2004;18:219-30.
30. Roy SS, Chakraborty P, Bhattacharya S. Intervention in cyclophosphamide induced oxidative stress and DNA damage by a flavonyl-thiazolidinedione based organoselenocyanate and evaluation of its efficacy during adjuvant therapy in tumor bearing mice. Eur J Med Chem. 2014;73:195-209.
31. Yuan D, Wang H, He H, et al. Protective effects of total flavonoids from Epimedium on the male mouse reproductive system against cyclophosphamide-induced oxidative injury by up-regulating the expressions of SOD3 and GPX1. Phytother Res. 2014;28:88-97.
32. Zarei M, Shivanandappa T. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of Decalepis hamiltonii in mice. Food Chem Toxicol. 2013;57:179-84.
33. Taskin E, Dursun N. Recovery of adriamycin induced mitochondrial dysfunction in liver by selenium. Cytotechnology. 2015;67:977-86.
34. Taskin E, Kindap EK, Ozdogan K, Aycan MB, Dursun N. Acute adriamycin-induced cardiotoxicity is exacerbated by angiotension II. Cytotechnology. 2016;68:33-43.
35. Taskin E, Ozdogan K, Kunduz KE, Dursun N. The restoration of kidney mitochondria function by inhibition of angiotensin-II production in rats with acute adriamycin-induced nephrotoxicity. Ren Fail. 2014;36:606-12.
36. Finn NA, Findley HW, Kemp ML. A switching mechanism in doxorubicin bioactivation can be exploited to control doxorubicin toxicity. PLoS Comput Biol. 2011;7:e1002151.
37. Gammella E, Maccarinelli F, Buratti P, Recalcati S, Cairo G. The role of iron in anthracycline cardiotoxicity. Front Pharmacol. 2014;5:25.
38. Ozdogan K, Taskin E, Dursun N. Protective effect of

carnosine on adriamycin-induced oxidative heart damage in rats. Anadolu Kardiyol Derg. 2011;11:3-10.

39. Dursun N, Taskin E, Ozturk F. Protection against adriamycin-induced cardiomyopathy by carnosine in rats: role of endogenous antioxidants. Biol Trace Elem Res. 2011;143:412-24.
40. Rashikh A, Abul KN, Akhtar M, Mahmood D, Pillai KK, Ahmad SJ. Protective effects of aliskiren in doxorubicin-induced acute cardiomyopathy in rats. Hum Exp Toxicol. 2011;30:102-9.
41. Ammar e, Said SA, El-Damarawy SL, Suddek GM. Cardioprotective effect of grape-seed proanthocyanidins on doxorubicin-induced cardiac toxicity in rats. Pharm Biol. 2013;51:339-44.
42. Matouk AI, Taye A, Heeba GH, El-Moselhy MA. Quercetin augments the protective effect of losartan against chronic doxorubicin cardiotoxicity in rats. Environ Toxicol Pharmacol. 2013;36:443-50.
43. Benguedouar L, Boussenane HN, Wided K, Alyane M, Rouibah H, Lahouel M. Efficiency of propolis extract against mitochondrial stress induced by antineoplasic agents (doxorubicin and vinblastin) in rats. Indian J Exp Biol. 2008;46:112-9.
44. Chenais B, Andriollo M, Guiraud P, Belhoussine R, Jeannesson P. Oxidative stress involvement in chemically induced differentiation of K562 cells. Free Radic Biol Med. 2000;28:18-27.
45. Aguirre L, Portillo MP, Hijona E, Bujanda L. Effects of resveratrol and other polyphenols in hepatic steatosis. World J Gastroenterol. 2014;20:7366-80.
46. Ko CC, Chen YJ, Chen CT, et al. Chemical proteomics identifies heterogeneous nuclear ribonucleoprotein (hnRNP) A1 as the molecular target of quercetin in its anti-cancer effects in PC-3 cells. J Biol Chem. 2014;289:22078-89.
47. Staedler D, Idrizi E, Kenzaoui BH, Juillerat-Jeanneret L. Drug combinations with quercetin: doxorubicin plus quercetin in human breast cancer cells. Cancer Chemother Pharmacol. 2011;68:1161-72.
48. Tabaczar S, Pieniazek A, Czepas J, Piasecka-Zelga J, Gwozdzinski K, Koceva-Chyla A. Quercetin attenuates oxidative stress in the blood plasma of rats bearing DMBA-induced mammary cancer and treated with a combination of doxorubicin and docetaxel. Gen Physiol Biophys. 2013;32:535-43.
49. Dogan Z, Kocahan S, Erdemli E, et al. Effect of chemotherapy exposure prior to pregnancy on fetal brain tissue and the potential protective role of quercetin. Cytotechnology. 2015;67:1031-8.
50. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. Antiartherosclerotic effects of plant flavonoids. Biomed Res Int. 2014;2014:480258.

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