

EXPERIMENTAL STUDY

Effects of quercetin on kidney injury induced by doxorubicin

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OBJECTIVES: The anthracycline antitumor drug doxorubicin causes severe nephrotoxicity in a variety of experimental animals and may be nephrotoxic to humans. The aim of present study was to determine the protective effects of quercetin against doxorubicin-induced kidney injury with light microscopy.

METHODS: Forty male Wistar albino rats were divided into four groups: control, doxorubicin, doxorubicin + quercetin and quercetin. A single dose of 20 mg/kg/ i.p. doxorubicin was used to induce injury. Quercetin was administered orally against doxorubicin toxicity. The kidneys were examined under light microscopy after H&E (hematoxylin-eosin) staining and the changes were scored.

RESULTS: Significant tissue injury was observed in doxorubicin-administered group. Among these injuries, renal tubular dilatation, tubular vacuolar changes, glomerular vacuolization, decrease in bowman space, bowman capsule thickening, and interstitial infiltration were evident. However, the injury induced by doxorubicin was attenuated with quercetin administration.

DISCUSSION: Quercetin decreased doxorubicin-induced kidney damage (Tab. 1, Fig. 4, Ref. 27). Textin PDF www.elis.sk.

KEY WORDS: doxorubicin, quercetin, nephrotoxicity, rat.

Introduction

Doxorubicin is an anthracycline which has been used in the treatment of cancer; however, it has nephrotoxic effects. Oxygen free radicals are produced in larger amounts due to the use of agents like doxorubicin, and this leads to oxidative damage in cellular membranes, organelles and even in genetic material (1). The most important indicators of this damage are lipid peroxidation and protein oxidation. Antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) are endogenous resources which can prevent this damage through removal of oxygen radicals.

The oxidant-antioxidant system has different sources, one of which is nitric oxide (NO). NO can be both a scavenger and a producer of free oxygen radicals. NO reacts with superoxide anions and is converted into peroxynitrite. Thus, peroxynitrite does both removes superoxide anion and becomes a damage-producing radical. Another source of the oxidant system is malondialdehyde (MDA), an indicator of lipid peroxidation. Other sources of radicals include neutrophils and myeloperoxidase (MPO) system. MPO production increases in inflammatory responses and produces hypochlorite radicals leading to cellular damage (2).

Flavonoid, plant pigments have been known for more than a century. Quercetin, a flavonol, is abundant in food, especially in onion. Tea is also rich in flavonol, particularly quercetin and kaempferol (3).

Recent studies have shown that flavonoids prevent DNA damage through certain mechanisms. They can be effective through protection and augmentation of endogenous oxidants. Most of flavonoids are able to activate glutathione-S-transferase (GST). Flavonoids including quercetin, myricetin and fisetin show their effects by significantly increasing GST activity (3-5).

The aim of this study was to investigate protective effects of quercetin against doxorubicin-induced renal injury under light microscope.

Material and methods

Forty male adult Wistar albino rats were used and divided into four groups: control (I; n = 10); doxorubicin (II; n = 10); doxorubicin + quercetin, (III; n = 10); quercetin (IV; n = 10). The control group received 0.5 ml 0.9 % NaCl intraperitoneal (i.p.) and 1cc 0.9 % NaCl orally for 10 days. The animals in the doxorubicin group were treated with a single 20 mg/kg/ i.p. dose of doxorubicin hydrochloride (C₂₇H₂₉NO₁₁HCl) (Doxo Teva 50 mg vial) on day 3 of the experiment. Group III received oral quercetin dihydrate (C₁₅H₁₀O₇.2H₂O; Quercetin dihydrate, Sigma) 50 mg/kg/day except on day 3 on which doxorubicin was applied. Group IV was administered oral quercetin dihydrate (C₁₅H₁₀O₇.2H₂O; Quercetin dihydrate, Sigma) 50 mg/kg/day for 10 days. After administration of the drugs, the rats were decapitated and their kidneys were removed and fixed in 10 % neutral-buffered formalin. Tissue samples were stained with H&E (hematoxylin-eosin) and histologically examined under light microscope.

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Light microscopic changes as tubular necrosis, atrophy, glomerular injury, vascular congestion, thrombosis, and interstitial inflammation were scored as follows: normal histology as 0 points; minimal changes as 1 point; moderate injury as 2 points; severe injury as 3 points.

Data were analyzed using SPSS for Windows v. 13.0 (Chicago, Ill., USA). For assessment of the homogeneity of groups, Shapiro-Wilk test was used. The comparison among groups was made with Kruskal–Wallis H Test. Mann–Whitney U Test is used for pairwise comparison of groups. $p < 0.05$ was considered significant.

Results

The control group revealed normal renal histology (Fig. 1). Nephrotoxic tissue damage due to doxorubicin is demonstrated in Figure 2. There was tubular necrosis and atrophy, vacuolization, glomerular damage, vascular congestion-thrombosis, and interstitial damage in the renal tissue in the doxorubicin group. Tissue morphology was improved with quercetin administration in Group III (Fig. 3). Quercetin administration alone caused no apparent changes in renal tissue (Fig. 4). All renal damage indicators, except for interstitial inflammation, significantly decreased in doxorubicin+quercetin group compared to doxorubicin group ($p < 0.05$) (Tab. 1). In addition, doxorubicin and doxorubicin+quercetin

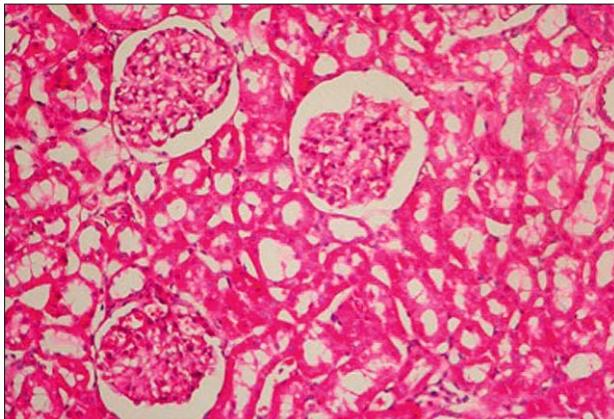


Fig. 1. Normal renal histology in control group.

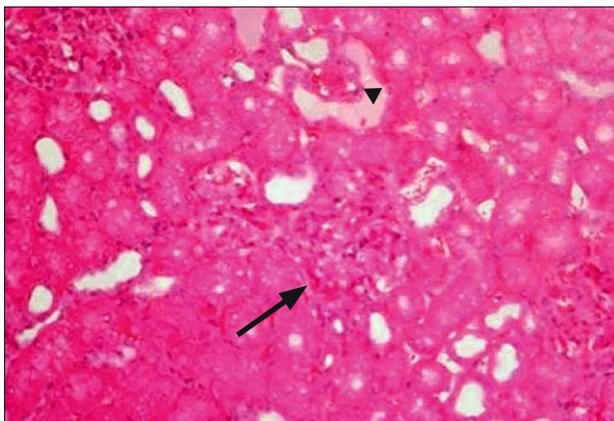


Fig. 2. Glomerular congestion (arrow) and eosinophilic material in a tubule (arrowhead) in Group II.

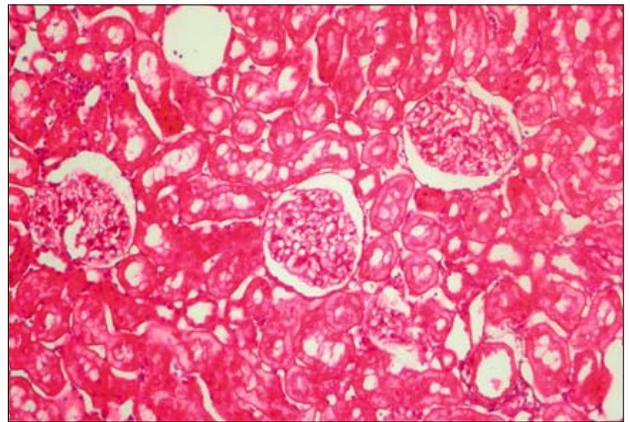


Fig. 3. Improved glomerular and tubular histology in Group III.

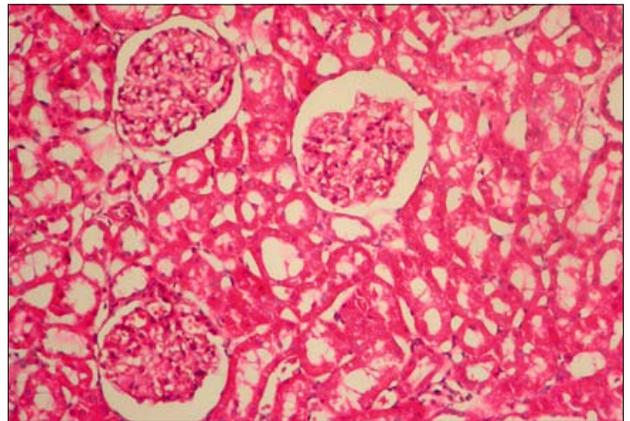


Fig. 4. Normal renal histology in Group IV.

groups yielded significantly increased pathological changes when compared to the control group ($p < 0.05$).

However, there was no significant difference between quercetin group and control group ($p > 0.05$), in terms of tissue damage (Tab. 1).

Discussion

Doxorubicin is used to treat solid tumors like carcinomas of the uterus, breasts, thyroid, esophagus, ovaries and lungs, acute leukemia, Hodgkin's and non-Hodgkin's lymphomas and soft tissue sarcomas (6, 7). Doxorubicin has been shown to cause renal toxicity due to oxidative stress in various experimental animal models and human studies (8, 9). As a result of oxygen use in aerobic organisms, reactive oxygen metabolites (ROM) occur naturally.

In a study by Liu et al, GSH, GST and SOD levels decreased in doxorubicin-administered rats (10). In a previous study conducted by our team, protective effects of caffeic acid phenethyl ester (CAPE) against doxorubicin-induced kidney injury were investigated. Histological examination showed glomerular vacuolization, tubular desquamation, adhesion, and thickening in Bowman's capsule and damage in the tubular and glomerular capillary basal membranes in doxorubicin group. Besides, the administration of CAPE combined with doxorubicin was observed to provide partial protection of the glomerules and tubules (11). Likewise, in the present study, we

Tab. 1. Statistical comparison of histological findings. Values are given as mean ± standard deviation.

Groups	Tubular necrosis-atrophy	Tubular vacuolization	Glomerular injury	Vascular congestion-thrombosis	Interstitial inflammation
I	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
II	2.20±0.13	2.50±0.16	2.20±0.13	2.00±0.21	1.80±0.20
III	1.40±0.16	1.40±0.16	1.40±0.16	1.20±0.13	1.30±0.15
IV	0.20±0.13	0.30±0.15	0.3±0.15	0.20±0.13	0.20±0.13
p value					
I and II	0.000	0.000	0.000	0.000	0.000
I and III	0.000	0.000	0.000	0.000	0.000
II and III	0.009	0.002	0.009	0.015	n.s.
I and IV	n.s.	n.s.	n.s.	n.s.	n.s.

Group I: Control, Group II: Doxorubicin, Group III: Doxorubicin+Quercetin, Group IV: Quercetin
n.s.: not significant

administered a single dose of doxorubicin 20 mg/kg into the peritoneum which resulted in similar tubular and glomerular damage.

Histological effects of doxorubicin nephrotoxicity were also shown by Jadhav et al (12). In another study by Lenderink et al., rats aged 8–10 weeks were administered a single dose of doxorubicin 10–11 mg/kg and sacrificed 4–5 weeks later. They observed glomerular sclerosis, tubular dilatation, and tubulointerstitial inflammation upon morphological examinations (12).

Weening et al reported that the rats administered with a single intravenous dose of doxorubicin 7.5 mg/kg had proteinuria at a mean amount of 98 mg, swelling in the glomerular epithelial cells, obliteration and vacuolization on morphological examinations (13). However, in the present study, we detected more severe glomerular and tubular damage with doxorubicin 20 mg/kg. The severity of histological injury may be attributable to the higher doses of doxorubicin in our study.

In a study by Dziegiel et al, acute toxicity occurred after administering a single intravenous dose of doxorubicin or daunorubicin. Additionally, subchronic toxicity was created by 3 doses (3 mg/kg) of the same antibiotics administered in one-week intervals. They observed glomerular sclerosis, glomerular vacuolization, tubular dilatation, tubular atrophy, interstitial lymphocytic infiltration, and material rich in protein in the tubular lumen in rats developing subchronic toxicity (14). In addition to the changes reported in the study by Dziegiel et al, in the present study we detected a reduction in Bowman's space and thickening in Bowman's capsule. This can be attributed to the dose we administered.

In a study by Erdogan et al, 0.6 mg/kg/day doxorubicin was administered to New Zealand rabbits for 6 days, while blood specimens were taken two hours after each administration, and histopathological examinations were performed. Doxorubicin-administered rabbits were observed to have increased serum urea and creatinine. In line with our report, their histopathological examination revealed degenerated tubular epithelium, large intertubular bleeding areas, glomerular atrophy, enlarged glomerular space, and material rich in protein in the tubular lumen (15).

In a study by El-Sheikh et al, doxorubicin caused dilatation in Bowman's space and marked degeneration of renal tubules that showed exfoliated cells, protein casts, and cystic dilatation (16).

Saad et al evaluated the effects of doxorubicin 25 mg/kg administration on heart, liver, and kidney tissues using biochemical and histopathological parameters and showed that focal tubular atrophy developed in the renal sections in doxorubicin-administered rats (17).

Boonsanit et al showed that intravenous administration of doxorubicin 7.5 mg/kg created nephrotoxic syndrome based on biochemical and histopathological parameters. They demonstrated glomerular capillary dilatation and tubular dilatation in sections of renal specimens (18).

In a study by Park et al., the rats were administered with a single dose of doxorubicin 7.5 mg/kg and sacrificed 21 hours later. Tubular dilatation, eosinophilic material in the tubular lumen, glomerular vacuolization, and thickening and adhesion in the Bowman's capsule were identified (19). In the present study, we also observed similar renal damage following an administration of a single dose of doxorubicin 20 mg/kg.

In the study by Mansour et al., an increase in serum urea levels and a sharp decrease in total protein and albumin levels were observed 24–48 hours after a single dose of doxorubicin 15 mg/kg had been administered. An increase in lipid peroxidation was also observed 24 hours after administration of doxorubicin. They claimed that captopril could be protective against nephrotoxicity created by doxorubicin (20). In a similar study by Bertani et al, proteinuria was detected 4–5 days after i.p. injection of a single dose of doxorubicin 7.5 mg/kg (21).

Flavonoids belong to polyphenolic compounds and can be found in all plants. Quercetin is one of the flavonoids known to have antioxidant features and achieve oxygen free radical uptake. Tea is rich in quercetin and kaempferol, which belong to flavonols and flavones (22).

Since quercetin was shown to have an antioxidant effect, we attempted to confirm the effect of quercetin in different doses on the damage created by doxorubicin. In literature, the protective effects of quercetin against different toxic agents were shown.

Behling et al administered a single dose of cisplatin 5 mg/kg into the peritoneum and gave quercetin 50 mg/kg through gavage 24 hours before administration of cisplatin. The administration of cisplatin was continued for 2, 5, and 20 days. They reported that cisplatin increased lipid peroxidation, urine volume, and plasma creatinine levels, while decreasing urine osmolality. However, those findings were reported to be reduced with quercetin (23).

Bongiovanni et al. used the plant flavonoids silymarin and quercetin to eliminate cytotoxicity caused by arsenite and made a conclusion that flavonoids taken through diet can be protective against this toxicity (24).

In a study by Ikizler et al, the protective effects of quercetin given as chronic treatment and acute infusion on ischemia-reperfusion were investigated. Final hemodynamic and biochemical pa-

rameters indicated a myocardial improvement in all stages of reperfusion in the quercetin group compared to the control group (25).

Polat et al investigated effects of intramuscular administrations of quercetin 50 mg/kg and desferrioxamine 100 mg/kg on renal damage created through hepatic ischemia-reperfusion. It was found that quercetin and desferrioxamine provided beneficial effects through modification of glutathione and MDA levels (26). In a similar study by Tokyol et al, reperfusion was created by 45-min hepatic ischemia, and the effects of intramuscular administrations of quercetin 50 mg/kg and desferrioxamine 100 mg/kg on liver damage were investigated. Plasma alanine aminotransferase levels, and MDA and glutathione activities were measured, and histopathological examinations of the obtained liver tissues were made. It was concluded that quercetin and desferrioxamine had a protective effect on the liver tissue against ischemia-reperfusion injury (27).

Conclusion

It can be concluded that in the light of histological findings, quercetin can have beneficial therapeutic effects in terms of prevention of renal damage caused by doxorubicin. However, detailed further studies with larger series are needed to confirm this conclusion.

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