Original Article

Quercetin Attenuating Doxorubicin Induced Hepatic, Cardiac and Renal Toxicity in Male Albino Wistar Rats

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ABSTRACT

Doxorubicin (DOX) is a broad spectrum anthracycline antibiotic used to treat multiple cancers. The use of DOX is limited by significant cardiotoxicity, hepatotoxicity and nephrotoxicity. Quercetin is a dietary-flavonoid found in plants which prevents oxidant injury and protects against lipid peroxidation. The objective of the present study was to assess the possible protective role of quercetin on doxorubicin induced hepatic, cardiac and renal toxicity in male Wistar rats. Doxorubicin (10mg/kg body weight) was administered twice and quercetin (100mg/kg body weight) was administered on all the 21 days of the experimental period. Body weight, heart weight, creatine kinase in serum and heart, the activities of the antioxidant enzymes in liver, heart and kidney were decreased in doxorubicin administered rats. Liver and kidney weight, liver marker enzymes in serum, liver and kidney, lactate dehyrogenase in serum, liver, heart and kidney, urea and creatinine in serum, lipid peroxide content in liver, heart and kidney were increased. All the biochemical parameters mentioned above were restored to near normal levels on quercetin administration with doxorubicin. Administration of doxorubicin in rats altered the biochemical parameters but, did not cause much effect on the tissues such as liver, heart and kidney which is evident with histological studies.

Keywords: Doxorubicin, Hepato toxicity, Cardiac toxicity, Renal toxicity, Quercetin, Protective effect.

INTRODUCTION

Doxorubicin is a potent anthracycline antineoplastic drug used to treat a wide range of malignancies[1]. However, the clinical use of DOX is limited by a significant dose-dependent cardiotoxicity, which may lead to end-stage heart failure[2]. In addition to DOX-induced cardiotoxicity, it also causes hepatotoxicity

and nephrotoxicity[3,4]. DOX-induced hepatotoxicity is generally mediated through the generation of free radicals[4]. DOX-induced nephrotoxicity causes increased capillary permeability and glomerular atrophy[3]. In addition to oxidative damage, DOX toxicity has been shown to induce inflammatory changes in the liver, heart and kidney tissues of DOX-administered rats[5].

The antitumor effects of plant flavonoids have been reported to induce cell growth inhibition and apoptosis in a variety of cancer cells[6]. Quercetin (3, 3´, 4´, 5, 7-penta hydroxyflavones), an important dietary flavonoid is found in a variety of plant-based foods such as red-onions, apples, tea, broccoli, capers, lovage, parsley, red grapes and a number of berries[7]. Quercetin exhibits antioxidant, anti-inflammatory and anticancer properties[8]. Studies had proved that quercetin treatment caused cell cycle arrests such as G2/M arrest or G1 arrest in different cell types[9,10]. Moreover, quercetin-mediated apoptosis may result from the induction of stress proteins, disruption of microtubules and mitochondrial release of cytochrome c and activation of caspases[11-13].

Doxorubicin is an antineoplatic anthracycline drug commonly used to treat multiple range of cancer. Quercetin, a dietary flavonoid found in many plants and vegetables. Quercetin attenuating the doxorubicin induced hepatic, cardiac and renal toxicity has not been proved yet in male albino Wistar rats. Hence the objective of this study is to assess the possible protective role of quercetin in ameliorating the toxicity induced by doxorubicin through biochemical and histological studies.

METHODS

Test materials

Doxorubicin was obtained from Samarth Life Sciences Private Limited, Mumbai. Quercetin was purchased from Hi-Media Laboratories Private Limited, Mumbai, India. All the other reagents and chemicals used were of analytical grade.

Experimental animals

Healthy adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing 230-250g were procured from Sri Raghavendra Enterprises, Bengaluru, India. The animals were housed in individual polypropylene cages (43×27×15cm) lined with paddy husk and maintained under controlled room temperature (23±2^{0}C) with alternating 12h of light and dark cycles. Rats were fed with standard rat pellet diet (Sai Enterprises, Chennai, India) and drinking water *ad libitum*. The experimental animals were acclimatized for 10 days prior to the treatment in the Animal House Facility, Pondicherry University, Puducherry.

The animal care and handling were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2008), Ministry of Environment and Forests, Government of India. The experimental animals were approved by the Institutional Animal Ethics Committee (IAEC), Pondicherry University, Puducherry (PU/IAEC/12/08).

Selection of doses, treatment and experimental design

Doxorubicin dosage (10mg/kg body weight dissolved in saline) was fixed based on the previous study carried out by Milic *et al*.,[14] and quercetin dosage (100mg/kg body weight dissolved in 0.5ml of saline) was selected based on the study of Guzy *et al*.,[15]. The experimental animals were divided into four groups of six animals each. **Group 1:** (Control) Saline was administered at a dosage of 0.5mL/kg body weight for 21 days orally through gavage. **Group 2:** (Quercetin) Quercetin was administered at a dosage of 100mg/kg body weight dissolved

in 0.5mL of saline orally through gavage. **Group 3:** (Doxorubicin) Doxorubicin was administered twice at a dosage of 10mg/kg body weight on first and tenth day of the treatment plan intraperitonially. **Group 4:** (Doxorubicin + Quercetin) Doxorubicin was administered as in group 3 and quercetin was administered as in group 2. After 30 minutes of DOX injection, quercetin was administered orally through gavage in group 3. The experimental period was for 21 days.

Body weight and organ weights

Body weight of each rat was measured on all the 21 days of the experimental period. The quantities of the test materials to be given were calculated daily based on the body weight of the experimental animals to ensure administration of the fixed dose. Body weight was also measured before euthanization of the experimental animals. Liver, heart and kidney (average of the two kidneys) were measured after euthanizing the animals.

Necropsy and sampling

The animals were fasted overnight (12 hours), weighed and euthanized using anesthetic ether on the day following the last dosing Latchoumycandane *et al*.[16]. Blood was collected by decapitation and allowed to clot and then centrifuged at 1500 rpm for 10 minutes at room temperature and serum was separated and used for the estimation of total protein, liver, heart and kidney marker enzymes. Liver, heart and kidney were surgically removed from the adhering connective tissues, washed in 1.15% KCl, weighed and stored at -80^{0}C until analysis. The liver, heart and kidney tissues (10% w/v) were homogenized in phosphate buffered saline (PBS, pH 7.4) using a Teflon homogenizer with the help of a glass homogenization tube. The tissue homogenates were then centrifuged at 10 000g for 10 minutes at 4^{0}C using a refrigerated eppendorf centrifuge. The clear supernatant obtained was used as enzyme source for assaying all the antioxidant parameter Muthu *et al*.[17].

Protein estimation

Concentration of protein in serum and tissue homogenates of liver, heart and kidney were estimated by the method of Lowry *et al*.,[18]. To 10µL of serum, liver, heart and kidney tissue homogenates, 5mL of alkaline copper reagent (Reagent C = 50mL of Reagent A + 0.5mL of Reagent B: Reagent A had 2% sodium carbonate and 0.1 N NaOH (w/v); Reagent B contained 0.5% copper sulphate in 1% sodium potassium tartarate). After adding reagent C, the reaction mixture was incubated at room temperature for 10 minutes. Then 0.5mL of 1N Folin's ciocalteau phenol reagent was added and incubated in dark for 20 minutes. A series of standards (Bovine serum albumin) in the range of 20-100µg were also treated in the same manner. The blue colour developed was read after 20 minutes at 660nm using a Shimadzu UV-Visible spectrophotometer. Values are expressed as mg/dL for serum and mg/g of tissue for liver, heart and kidney tissue homogenates.

Liver marker enzymes

Alkaline phosphatase (ALP)

Activity of ALP was determined by the method of King and Armstrong[19]. 10mL of buffered substrate (0.127g of disodium phenyl phosphate in 50mL of distilled water, to which 50mL of phosphate buffer (pH 10) was added) was incubated at 37^{0}C for 5 minutes. Then 0.5mL of serum was added, mixed well and incubated for 30 minutes at 37^{0}C. A control having 10.5mL of buffered substrate was also run. After incubation, 4.5mL of 1N Folin's ciocalteau phenol reagent (FCPR) was added and centrifuged

at 5000rpm for 5 minutes. To 10mL of supernatant, 2.5mL of 10% sodium carbonate was added. Simultaneously, standards were also prepared using varying concentration of standard phenol (10µg/mL-phenol was diluted with 0.1N HCl) to which FCPR and sodium carbonate were added as above to compare the results. Activity of ALP was calculated from the difference between test and control. Values are expressed as IU/L.

Aspartate transaminase & Alanine transaminase (AST & ALT)

Activity of AST and ALT were estimated by the method of Reitman and Frankel[20]. 0.5mL of substrate (for AST, 2.66g of DL-aspartate and 30mg of α-ketoglutarate were dissolved in 20.5 mL of 1M NaOH, pH was adjusted to 7.4 and made up to 100mL; for ALT, 1.78g of DL-alanine and 30mg of α-ketoglutarate were dissolved in 20mL of phosphate buffer containing 1.25mL of 0.4M NaOH, pH was adjusted to 7.4 and made up to 100mL with phosphate buffer) was incubated at 37^0C for 5 minutes. 0.1mL of serum was added and incubated at 37^0C for 1 hour for AST and 30 minutes for ALT. Standards were prepared by using 2mM pyruvate/mL in phosphate buffer. After incubation, the reaction was arrested by adding 0.5mL of 2, 4-dinitorphenyl hydrazine (DNPH) and incubated at room temperature for 20 minutes. Then 5mL of 0.4M NaOH was added and left for 10 minutes at room temperature. The absorbance was read at 420nm. Values are expressed as IU/L.

Creatine kinase

Creatine kinase in serum and heart were determined by the kit method supplied by Agappe Diagnostics Ltd, Kerala, India. Values are expressed as mg/dL.

Lactate dehydrogenase

The activity of lactate dehydrogenase was estimated by the method of Goldberg and Hawtrey[21]. To 2.5mL of Tris-EDTA-NADH buffer pH 7.4, 300µL of enzyme source was added and the contents of the reaction tube were mixed well. The tubes were then incubated at 37ºC for 15 minutes. This incubation permits reduction of any pyruvate by NADH and other oxo-acids present in the enzyme source. At the end of incubation, 200µL of pyruvate solution was added to the reaction sample and the sample was transferred to a 3mL cuvette. The change in the absorbance due to dehydrogenation of NADH was noted for 5 minutes at 340 nm using a spectrophotometer. Values are expressed as U/L.

Urea

Urea was estimated by Berthelot *et al*.,[22] method. To 1mL of serum, 2mL of acid reagent (0.25 mL of reagent A was mixed with 500 mL of reagent B, reagent A: 50mg of ferric chloride, 0.2mL of distilled water, 1mL of O-phosphoric acid and 2.5mL of distilled water; reagent B: 50mL of concentrated sulphuric acid and 450mL of distilled water) and 2mL of DAM-TSC (33.5 mL of reagent C was mixed with 33.5mL of reagent D and made up to 500mL with distilled water, reagent C: 1g of diacetyl monoxime in 50mL of distilled water; reagent D: 250mg of thiosemicarbazide in 50mL of distilled water) reagent were added. The contents of the tube were mixed well and heated in a boiling water bath for 20 minutes. The tubes were then cooled to room temperature and the pink color developed was read against a blank at 520nm within 15 minutes. The amount of urea in serum is expressed as milligram per 100mL.

Creatinine

Creatinine was estimated by alkaline picrate method which makes use of Jaffe's reaction[23]. To 1mL of serum, 2mL of distilled water was added. 1mL of 1% picric acid and 1mL of 0.75N NaOH were then added. The tubes were incubated for 15 minutes at room temperature and read at 540nm. The amount of creatinine in serum is expressed as milligram per 100mL.

Lipid peroxidation

Lipid peroxide content of the liver, heart and kidney tissue samples were determined by Thiobarbituric acid reactive substances (TBARS)[24]. The tissues were homogenized with Tris-HCl buffer (pH 7.5, 0.025M). To 1 mL of tissue homogenate, 2 mL of Thiobarbituric acid – Trichloroactetic acid – HCl (TBA-TCA-HCl: 1:1:1 w/v) was added and kept in a boiling water bath for 30 minutes. TBA reacts with aldehydes at 100° C under acidic condition. The tubes were then cooled and centrifuged at 2000rpm for 10 minutes. A pink colored complex formed in the supernatant was read at 535 nm. A series of standards in the range of 2 – 10 nmoles were treated in a similar manner. The enzyme activity was expressed as millimoles of malondialdehyde produced per minute per mg of protein.

Antioxidant parameters

Catalase

Catalase was assayed by the method of Claiborne[25] with slight modifications. Briefly, the assay mixture contained 2mL of phosphate buffer (50mM, pH 7.0), 50µL of 0.17% H_2O_2 and 30µL of enzyme source. The decrease in absorbance was read immediately at 240nm against a blank containing all the components except the enzyme source at 10-s intervals for 3 minutes on a Shimadzu UV-visible spectrophotometer. Activity of the enzyme is expressed as micromoles of hydrogen peroxide consumed per minute per milligram of protein.

Superoxide dismutase

Superoxide dismutase was assayed by the method of Marklund and Marklund[26]. Briefly, the assay mixture contained 2.5mL of 50mM tris-HCl buffer containing 1mM EDTA (pH 7.6), 300µL of 0.2mM pyrogallol and 300µL of enzyme source. The increase in absorbance was read immediately at 420nm at 10-s intervals for 3 minutes against a blank containing phosphate buffer. Activity of the enzyme is expressed as micromoles of pyrogallol oxidized per minute and milligram of protein.

Glutathione reductase

Glutathione reductase was assayed by the method of Carlberg and Mannervik[27]. Briefly, the assay mixture contained 1.75mL of phosphate buffer (100mM, pH 7.6), 100µL of 0.2µM NADPH, 100µL of 10mM EDTA, 50µL of 20mM oxidized glutathione and 50µL of enzyme source. Disappearance of NADPH was read immediately at 340nm against a blank containing all the components except the enzyme at 10-s intervals for 3 minutes. Activity of the enzyme is expressed as nanomoles of NADPH oxidized per minute per milligram of protein.

Glutathione peroxidase

Glutathione peroxidase was assayed by the method of Paglia and Valentine[28]. Briefly, the assay mixture contained 1.59mL of phosphate buffer (100mM, pH 7.6), 100µL of 10mM EDTA, 100µL of 0.065% sodium azide, 50µLof 0.62% glutathione reductase, 100µL of 0.03% reduced glutathione, 100µL of 0.2µM NADPH, 10µL of 19mM hydrogen peroxide, 10µL of enzyme source. Disappearance of NADPH

was read immediately at 340nm against a blank containing all the components except the enzyme at 10-s intervals for 3 minutes. Activity of the enzyme is expressed as nanomoles of NADPH oxidized per minute per milligram of protein.

Histometric studies

A portion of liver, heart and kidney were fixed in 10% neutral phosphate buffered formalin (pH 6.8) for 72 hours. The tissues were processed by standard histopathological techniques using graded ethanol, cleared with xylene and embedded in paraffin wax. Paraffin sections were cut at 5µm thickness and stained with hematoxylin and eosin for light microscopy examination.

Statistical analysis

Data are expressed as mean ± SD for four animals per group since two rats in group 3 and one animal in group 4 died. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-test using SPSS version 16.0 for Windows. Data are statistically significant at $P \leq 0.05$.

RESULTS

Clinical observation and mortality

The general appearance, behavior and mortality were observed in all the groups of experimental animals during the treatment period. A red colored lesion was observed at the site of injection in DOX treated animals and red exudates accumulation around the eyes was observed in both DOX and DOX+QUE treated animals, although more extensively in DOX treated rats. DOX administered animals appeared to be sicker, weaker and lethargic when compared with DOX+QUE treated animals. The urine was pink colored on the day of doxorubicin administration. Two animals: one animal on the 12th day and the other animal on the 13th day died during the experimental period in doxorubicin treated group (group 3). One animal in doxorubicin + quercetin treated group (group 4), died on 19th day of the experimental period. However, no mortality was observed after the first dosage of DOX. There were no changes observed in general appearance and behavior of control and quercetin treated rats, also no mortality was observed in control and QUE treated animals.

Body weight and organ weights

The body weight in the doxorubicin treated group decreased significantly when compared to control and quercetin treated rats. In DOX+QUE treated group the body weight was initially decreasing and reached to near normal level after 14th day of the experimental period. The body weight in control and quercetin treated animals increased consistently and no decrease in body weight was observed throughout the experimental period (Table 1). The heart weight was found to be decreased, liver and kidney weights were found to be increased in DOX treated rats and no significant changes in the organ weights were observed in rest of the group of animals (Table 2).

Protein concentration in the serum, liver, heart and kidney tissues

The protein concentration in the serum was found to be decreased in DOX treated rats and the protein concentration was increased to near normal levels on DOX+QUE treatment. The total protein concentration in liver, heart and kidney were found to be increased in DOX treated animals and the level was restored in DOX+QUE treated group (Table 3). No significant changes were observed in protein concentration in serum, liver, heart and kidney in quercetin and control animals.

Liver, heart and kidney bio-markers

The liver marker enzymes such as alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) in serum, liver and kidney (Table 4) was elevated in DOX treated rats. Significant changes were observed in DOX+QUE treated group when compared to control and DOX treated groups. The cardiac biomarker creatine kinase (CK) was found to be decreased in DOX treated group and activity of CK was restored in DOX+QUE treated rats in the serum and heart (Table 5). QUE treated group showed normal activity of CK. The activity of lactate dehydogenase (LDH) was increased in serum, liver, heart and kidney in group 3 and LDH activity was restored in group 4 (Table 6). The kidney biomarkers such as urea and creatinine in serum were increased in group 3 when compared to the control group (Table 7). The hepatic, cardiac and renal bio-markers were restored to near normal level in DOX+QUE treated animals and no significant change was observed in QUE treated rats when compared to control rats.

Lipid peroxide content and antioxidant enzymes

Lipid peroxidation was found to be increased in serum, liver, heart and kidney in group 3. The levels were close to normal in DOX+QUE treated group (Table 8). The antioxidant enzymes activities such as CAT, SOD, GPX and GR decreased in liver, heart and kidney tissues in DOX treated groups and were restored in DOX+QUE treated groups (Table 9). No significant change in lipid peroxide content and antioxidant enzymes were observed in the rats treated with QUE when compared to control animals.

Histopathological examination

Liver

Control group animals showed normal architecture. QUE treated group showed normal architecture and non-specific lymphoid infiltrate in the lobules. DOX treated group depicted focal hepatocytic necrosis and regenerative changes with normal liver architecture. Mild hepatotoxicity was observed and some hepatocytes showed regeneration. DOX+QUE treated group illustrated protective effect and normal architecture was observed (Figure 1).

Heart

Control group animals showed normal architecture. QUE treated group illustrated normal architecture within normal limits. DOX treated group demonstrated evidence of injury and syncytial giant cells and regeneration was observed in some of the cardiocytes, also some focal cells showed cytoplasmic vacuolation. DOX+QUE treated group depicted normal architecture as control group (Figure 2).

Kidney

Control group animals showed normal morphology. Cortex and medulla showed glomeruli and tubules and interstitium within normal limits. Calyces were lined by transitional epithelium. QUE treated group described transitional epithelium within normal limits. DOX treated group did not show any effect. Very mild damage was observed which is considered not toxic. DOX+QUE treated group illustrated normal morphology (Figure 3).

DISCUSSION

The general appearance and behavior changes observed in the present study were in parallel with the studies conducted by

Siveski-Iliskovic *et al*.,[29]. 33% mortality was observed in the present study in DOX treated group animals. 16% mortality was observed in DOX+QUE treated rats. The liver weight in the DOX administered animals increased may be due to oxidative stress. The decrease in body weight, heart and kidney weights in DOX treated rats may be due to decreased food intake. This is analogous to the study done by Beshay *et al*.,[1] which demonstrates that DOX-treated animals lost an average of 5% body weight when compared with the controls and food intake in DOX treated animals was significantly reduced by almost 50% from the control.

The total protein concentration in the serum diminished on DOX administration and co-administration of QUE with DOX restored the levels to normal as in control group. However, QUE and control animals did not show significant variation. This observation is concordant with the earlier report by Mansour *et al*.,[30]. The reduced level of protein concentration in the serum might be due to decreased food intake by the DOX treated rats and thereby reduction in the rate of protein synthesis. Hepatic, cardiac and renal protein concentration might have increased due to oxidative stress and depletion of the intrinsic antioxidant machinery. QUE administration with DOX brought back the protein concentration by re-establishing the antioxidant system in liver, heart and kidney.

The liver bio-markers ALP, AST and ALT in serum, liver and kidney increased in DOX treated group. This result is comparable to the observation made by Wang *et al*.,[31] that reveals the acute hepatotoxicity of DOX (20mg/kg; single dose) by an increase in serum biochemical markers such as ALT and AST in C57BL/6 mice. Quercetin supplementation did not exhibit significant change in the activities of ALT and AST. Conversely, the co-administration of DOX and quercertin (100mg/kg) resulted in a partial reversal of DOX-induced serum increase in ALT and ALP[31]. Hence quercetin decreases DOX-induced acute hepatotoxicity in normal mice. Our results depict the same and the increase in the liver bio-markers ALP, AST and ALT is characteristic of hepatocellular and decreased liver functions as demonstrated by Alshabanah *et al*.,[32]. Hypoalbuminemia in DOX treated rats was observed in the present study, which is a characteristic feature of nephrotic syndrome as described in the previous studies[30,33].

Heart is very sensitive to reactive oxygen species (ROS) induced damage because of its highly oxidative metabolism and fewer antioxidant defenses compared to other organs. Hence, increase in the activities of LDH and CK in the serum of the animals treated with doxorubicin is expected[34]. Serum LDH and CK are considered important markers of early and late cardiac injury especially during clinical follow-up of doxorubicin therapy[33]. Previous studies have demonstrated similar elevations in cardiac enzymes activities in rats following challenge with a single cumulative dose of doxorubicin (15-20 mg/kg)[36-38]. The present study shows the fact that DOX administration had caused an increase in LDH activity and CK activity was decreased in serum and heart. Since, LDH is released during tissue injury, an increased activity of LDH in serum and heart in the present study reveals damage in the heart and hence got accumulated in the serum. CK had been utilized in combating the oxidative stress created by DOX which is an Adenosine Triphosphate (ATP) dependent enzyme. Hence there is a decrease in CK activity in serum and heart of the DOX treated animals. Elevated levels of serum urea and creatinine in DOX treated rats might be due to intrinsic acute renal failure[30,39]. However, co-administration of

QUE with DOX restored the activities of LDH and CK and also the levels of serum urea and creatinine which might be due to the antioxidant potential of quercetin.

The lipid peroxide content in liver, heart and kidney increased in DOX treated rats. An altered membrane function on DOX induced lipid peroxidation is held responsible for the ECG changes, most notably ST segment prolongation[40]. Thus, membrane stabilization would affect the propagation phase of lipid peroxidation, in that the mobility of lipid peroxy radicals would be prevented in interacting with the adjacent membrane polyunsaturated fatty acids[41]. Lipid peroxidation is the main cause of DOX-induced nephrotoxicity and that doxorubicin-induced lipid peroxidation is probably due to depletion of non-protein sulfhydryl containing compounds[30]. Yagamurca *et al*.,[38] reported that single dose of DOX (20mg/kg) resulted in renal lipid peroxidation in male Sprague-Dawley rats.

Oxidative stress is associated with DOX-induced cell injury and DNA damage. DOX induces hepatic dysfunction by changing the activities of superoxide dismutase, catalase and glutathione (GSH) enzymes in liver tissues[4]. The activities of the antioxidant enzymes CAT, SOD, GPx and GR in DOX administered rats decreased in the present study revealing the damage in the liver antioxidant machinery. Quercetin also exhibits a protective effect against drug toxicity, which induces oxidative stress. For example, quercetin prevents epirubicin-induced acute oxidative stress in rat liver cells and mitochondria[42]. Quercetin also increases GSH levels in rats under long-term alcohol consumption-induced oxidative stress[43]. Ayle *et al*.,[44] demonstrated a decrease in renal CAT, GPx and GSH activities 10 days after DOX administration (20mg/kg; single injection) in Male Wistar-albino rats. The present study shows similar decrease in the antioxidant enzymes CAT, SOD, GPx and GR in the kidney. The fall in antioxidant enzymes level in kidney in DOX treated group might be due to an increase in lipid peroxidation[39]. Mesbah *et al*.,[45] recorded an increase in renal glutathione and decreased MDA levels in groups of animals pretreated with flavonoids of propolis (100mg/kg body weight) that revealed the preventive effects of flavonoids against renal oxidative stress induced by DOX. Hence the flavonoid QUE (100mg/kg body weight) might exhibit the preventive effect in combating the renal oxidative stress caused by DOX.

DOX administration notably decreased the cardiac CAT, SOD and GSH activities associated with an increase in cardiac lipid peroxidation[46]. Though the exact mechanism(s) whereby doxorubicin would induce cardiac toxicity is not fully explored, the principal mechanism could possibly be through free radical generation by the "redox-cycling" of the anthracycline molecule and/or by the formation of anthracycline-iron complexes[47]. The cardioprotective effects of the flavonoid have been attributed to its antioxidant and iron chelating properties[46]. Hence QUE shows protective effects against DOX induced hepatic, cardio and renal toxicity by its antioxidant and iron chelating properties.

Inspite of the altered levels in the biochemical parameters, histological examination does not reveal much toxicity in this study when compared to earlier studies done on quercetin attenuation on doxorubicin in mice[29]. This might be due to the difference in intraperitoneal doses and treatment schedule. DOX administration twice intraperitoneally in rats in the present study caused variations in the biochemical parameters in serum, liver, heart and kidney. However, DOX caused mild toxicity which were within normal limits and considered non-toxic in the histological studies of liver, heart and kidney sections of rats.

CONCLUSION

The present study demonstrates that doxorubicin administration twice i.e. on the first and the tenth day of the experimental period (21 days) at a dosage of 10mg/kg body weight of male Wistar-Albino rats caused acute hepatic, cardiac and renal injury. Besides, this study revealed that co-administration of quercetin at a dosage of 100mg/kg body weight of the rat protected liver, heart and kidney tissues from DOX induced toxicity. Preventive effects of quercetin on hepatic, cardiac and renal lesions may be due to its antioxidant property. Although, the exact mechanisms remain to be clarified, quercetin could be an effective route of therapy to enhance therapeutic efficacy and to reduce DOX induced toxicity in clinical chemotherapy.

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Table 1. Effect of quercetin on body weight in the rats treated with doxorubicin

Group	Initial body weight (g)	Final body weight (g)	Weight gain (g)
Group 1 (Control)	252.00 ± 22.05	273.05 ± 18.30	15.00 ± 1.00
Group 2 (Quercetin)	249.00 ± 18.37	265.04 ± 24.58	13.00 ± 1.26
Group 3 (Doxorubicin)	249.67 ± 19.25	213.04 ± 13.91[a]	-36.67 ± 1.05[a]
Group 4 (Doxorubicin + Quercetin)	250.00 ± 14.32	225.00 ± 20.63[b]	-25.00 ± 2.13[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin

Table 2. Effect of quercetin on organ weights in the rats treated with doxorubicin

Group	Liver weight (g)	Heart weight (g)	Kidney weight (g) (Average of both the kidneys)
Group 1 (Control)	7.97 ± 0.77	0.86 ± 0.00	0.85 ± 0.04
Group 2 (Quercetin)	7.63 ± 0.50	0.81 ± 0.04	0.84 ± 0.08
Group 3 (Doxorubicin)	8.71 ± 0.64[a]	0.63 ± 0.03[a]	0.92 ± 0.01[a]
Group 4 (Doxorubicin +Quercetin)	7.13 ± 0.00[b]	0.77 ± 0.00[b]	0.77 ± 0.00[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin

Table 3. Effect of quercetin on total protein in serum, liver, heart and kidney in the rats treated with doxorubicin

Group	Serum (g/dL)	Liver (mg/g of tissue)	Heart (mg/g of tissue)	Kidney (mg/g of tissue)
Group 1 (Control)	5.01 ± 0.15	77.88 ± 7.66	55.24 ± 3.89	107.27 ± 4.84
Group 2 (Quercetin)	5.19 ± 0.12	72.63 ± 7.09	56.25 ± 2.67	109.32 ± 8.79
Group 3 (Doxorubicin)	3.27 ± 0.30[a]	88.87 ± 5.93[a]	77.23 ± 6.79[a]	125.90 ± 1.90[a]
Group 4 (Doxorubicin +Quercetin)	3.87 ± 0.00[b]	79.37 ± 0.00[b]	66.83 ± 5.28[b]	114.90 ± 0.00[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin

Table 4. Effect of quercetin on ALP, AST and ALT activities in serum, liver and kidney homogenate of the rats treated with doxorubicin

	Group	ALP (U/L)	AST (U/L)	ALT (U/L)
Serum	Group 1 (Control)	40.31 ± 3.16	79.71 ± 1.42	42.25 ± 2.15
	Group 2 (Quercetin)	40.26 ± 3.84	75.63 ± 3.29	40.02 ± 0.92
	Group 3 (DOX)	36.38 ± 0.12[a]	62.26 ± 0.74[a]	36.57 ± 2.25[a]
	Group 4 (DOX+QUE)	38.04 ± 0.00[b]	72.04 ± 0.00[b]	42.45 ± 0.00[b]
Liver	Group 1 (Control)	30.76 ± 2.22	49.10 ± 2.29	94.20 ± 0.24
	Group 2 (Quercetin)	37.56 ± 3.04	49.38 ± 2.04	95.09 ± 0.87
	Group 3 (DOX)	46.48 ± 1.15[a]	63.93 ± 0.99[a]	116.23 ± 2.22[a]
	Group 4 (DOX+QUE)	40.83 ± 0.00[b]	54.24 ± 0.00[b]	105.17 ± 8.03[b]
Kidney	Group 1 (Control)	57.65 ± 4.78	52.98 ± 2.93	35.43 ± 2.06
	Group 2 (Quercetin)	55.23 ± 4.89	56.46 ± 3.63	38.22 ± 1.04
	Group 3 (DOX)	74.35 ± 7.54[a]	69.24 ± 6.05[a]	55.58 ± 3.28[a]
	Group 4 (DOX+QUE)	68.05 ± 3.07[b]	59.64 ± 4.09[b]	48.27 ± 2.19[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin
(DOX – Doxorubicin; DOX+QUE – Doxorubicin + Quercetin)

Table 5. Effect of quercetin on creatine kinase (CK) level in serum and heart of the rats treated with doxorubicin

Group	Serum (U/L)	Heart (U/L)
Group 1(Control)	536.47 ± 36.17	544.76 ± 50.32
Group 2 (Quercetin)	519.58 ± 27.23	540.48 ± 30.95
Group 3 (Doxorubicin)	274.45 ± 16.04[a]	222.49 ± 22.00[a]
Group 4 (Doxorubicin + Quercetin)	427.94 ± 27.09[b]	460.00 ± 39.01[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin

Table 6. Effect of quercetin on lactate dehydrogenase in serum, liver, heart and kidney of the rats treated with doxorubicin

Group	Serum (U/L)	Liver (U/L)	Heart (U/L)	Kidney (U/L)
Group 1 (Control)	138.78 ± 12.03	17.48 ± 1.06	10.22 ± 1.07	13.53 ± 1.35
Group 2 (Quercetin)	147.37 ± 11.26	15.27 ± 1.44	9.87 ± 0.38	16.16 ± 1.14
Group 3 (Doxorubicin)	185.55 ± 16.65[a]	33.55 ± 0.97[a]	25.08 ± 1.52[a]	29.59 ± 1.59[a]
Group 4 (Doxorubicin + Quercetin)	174.67 ± 13.24[b]	26.19 ± 1.80[b]	18.37 ± 0.93[b]	26.97 ± 1.99[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin

Table 7. Effect of quercetin on serum urea and creatinine of the rats treated with doxorubicin

Group	Urea (mg/dL)	Creatinine (mg/dL)
Group 1 (Control)	0.42 ± 0.02	0.47 ± 0.04
Group 2 (Quercetin)	0.39 ± 0.02	0.42 ± 0.04
Group 3 (Doxorubicin)	0.65 ± 0.03[a]	0.81 ± 0.06[a]
Group 4 (Doxorubicin + Quercetin)	0.57 ± 0.04[b]	0.64 ± 0.03[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin

Table 8. Effect of quercetin on lipid peroxide content by thiobarbituric acid reactive substances (TBARS) in liver, heart and kidney of the rats treated with doxorubicin

Group	Liver (mM of MDA/mg protein)	Heart (mM of MDA/mg protein)	Kidney (mM of MDA/mg protein)
Group 1 (Control)	12.38 ± 1.04	1.34 ± 0.12	2.98 ± 0.19
Group 2 (Quercetin)	11.89 ± 0.90	1.33 ± 0.13	2.67 ± 0.17
Group 3 (Doxorubicin)	29.32 ± 1.67[a]	3.23 ± 0.13[a]	4.34 ± 0.11[a]
Group 4 (Doxorubicin + Quercetin)	18.37 ± 0.71[b]	1.62 ± 0.10[b]	3.98 ± 0.16[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin, MDA - Malondialdehyde

Table 9. Effect of quercetin on catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase in liver, heart and kidney homogenate of the rats treated with doxorubicin

	Group	CAT (Units[1]/mg protein)	Total SOD (Units[2]/mg protein)	GR (Units[3]/mg protein)	GPx (Units[4]/mg protein)
Liver	Group 1 (Control)	75.47 ± 6.72	5.12 ± 0.03	52.20 ± 1.05	52.06 ± 4.29
	Group 2 (Quercetin)	62.63 ± 3.51	5.10 ± 0.02	50.77 ± 3.77	45.11 ± 2.73
	Group 3 (DOX)	26.30 ± 1.38[a]	3.09 ± 0.10[a]	44.31 ± 4.44[a]	27.58 ± 2.02[a]
	Group 4(DOX+QUE)	55.56 ± 2.09[b]	4.11 ± 0.00[b]	49.06 ± 0.00[b]	44.65 ± 0.00[b]
Heart	Group 1 (Control)	16.23 ± 1.32	6.18 ± 0.57	10.32 ± 1.01	5.93 ± 0.34
	Group 2 (Quercetin)	15.87 ± 0.87	6.87 ± 0.55	9.73 ± 0.07	5.78 ± 0.24
	Group 3 (DOX)	6.32 ± 0.54[a]	4.29 ± 0.40[a]	6.28 ± 0.05[a]	1.99 ± 0.09[a]
	Group 4(DOX+QUE)	9.87 ± 0.89[b]	6.36 ± 0.46[b]	7.23 ± 0.04[b]	3.98 ± 0.33[b]
Kidney	Group 1 (Control)	126.55 ± 10.81	4.28 ± 0.06	57.84 ± 0.43	12.29 ± 1.09
	Group 2 (Quercetin)	135.69 ± 12.75	4.07 ± 0.01	54.44 ± 1.12	13.73 ± 1.16
	Group 3 (DOX)	106.51 ± 8.06[a]	2.06 ± 0.02[a]	28.45 ± 2.09[a]	8.24 ± 0.47[a]
	Group 4(DOX+QUE)	119.64 ±10.00[b]	3.33 ± 0.02[b]	39.38 ± 2.89[b]	13.72 ± 1.23[b]

(Values are mean ± SD for 4 rats in each group)
P values ≤ 0.05; compared with [a] control and [b] doxorubicin; (DOX – Doxorubicin; DOX+QUE – Doxorubicin + Quercetin)
1–µmoles of H_2O_2 consumed per minute
2– µmoles of pyrogallol oxidized per minute
3&4– nmoles of NADPH oxidized per minute

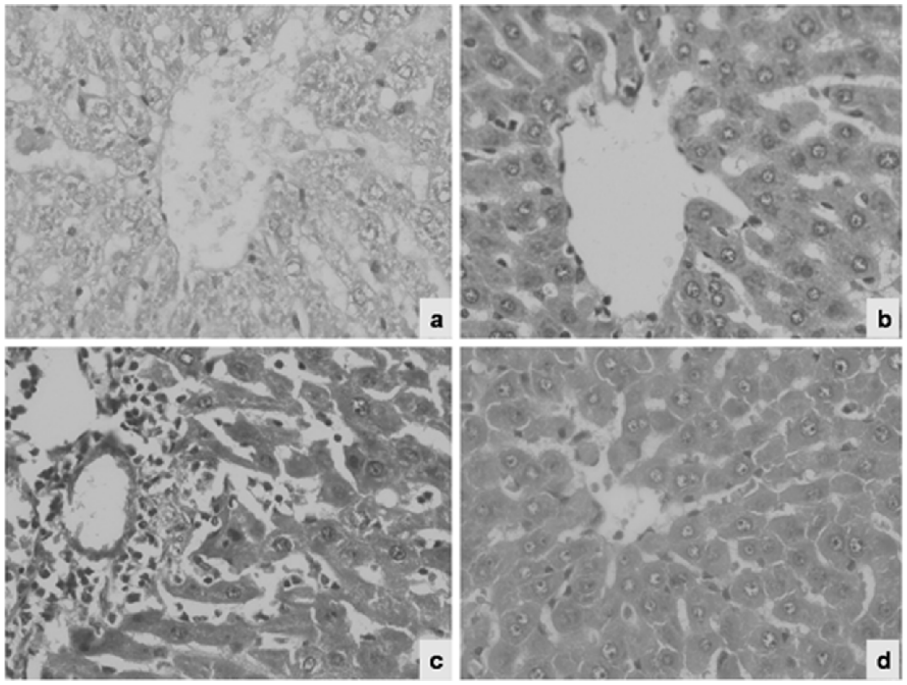

Figure 1. Light microscopic examination of the liver section of the rats at 40X (Hematoxylin and Eosin; H & E stained). Control group showing normal architecture (a). QUE treated groups showing normal architecture and non-specific lymphoid infiltrate in the lobules (b). DOX treated group depicting focal hepatocytic necrosis and regenerative changes showing normal liver architecture, also mild hepatotoxicity and some hepatocytes describing regeneration (c). DOX+QUE treated group illustrating protective effect and normal architecture (d)

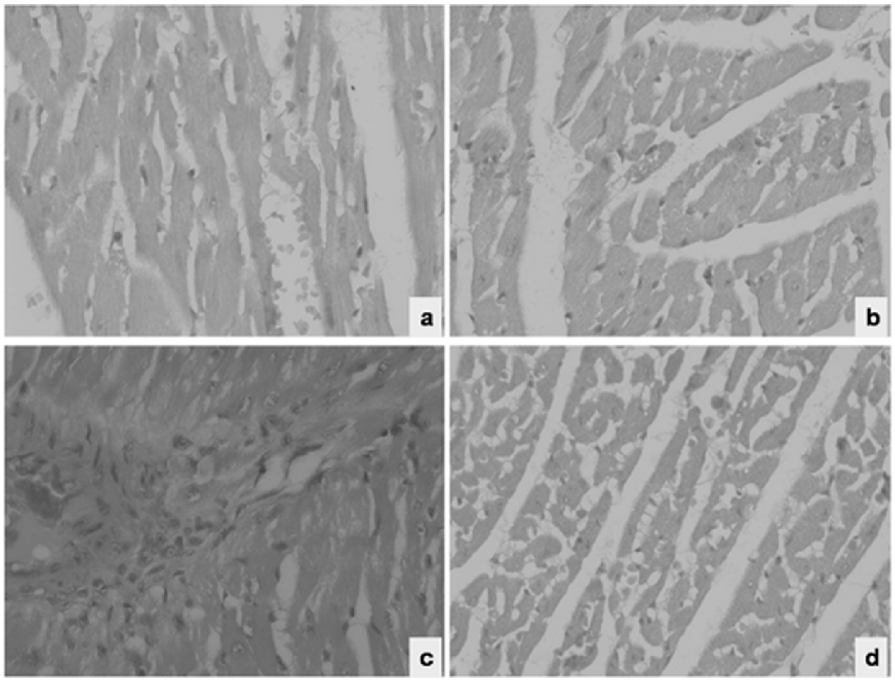

Figure 2. Light microscopic examination of the heart section of the rats at 40X (Hematoxylin and Eosin; H & E stained). Control group illustrating normal architecture (a). QUE treated group showing normal architecture within normal limits (b). DOX treated group describing evidence of injury and syncytial giant cells and regeneration is observed in some of the cardiocytes, also some focal cells depicting cytoplasmic vacuolation (c). DOX+QUE treated group representing normal architecture as control group (d)

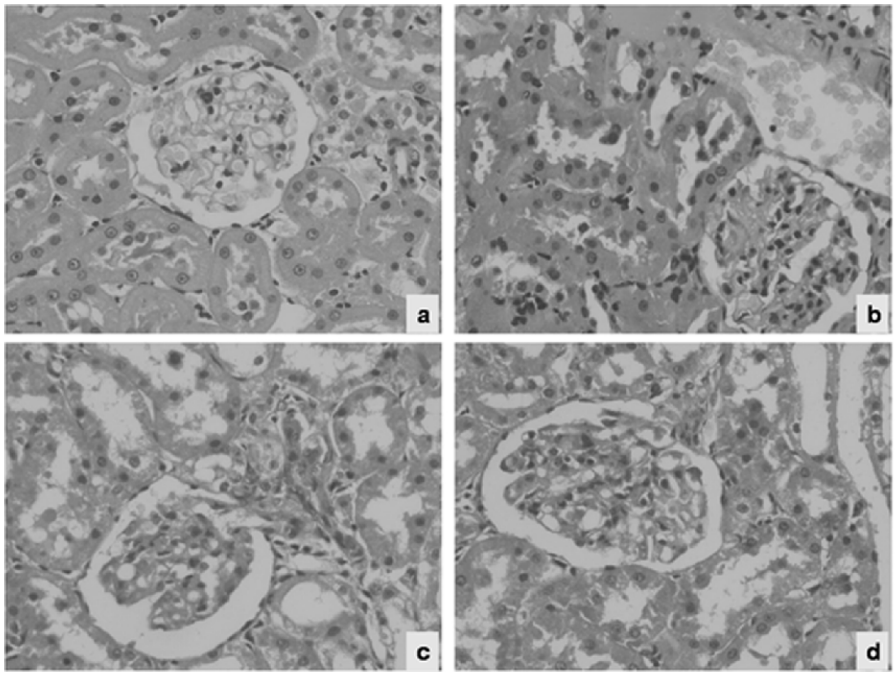

Figure 3. Light microscopic examination of the kidney section of the rats at 40X (Hematoxylin and Eosin; H & E stained). Control group showing normal morphology: Cortex and medulla showing glomeruli and tubules and interstitium within normal limits. Calyces are lined with transitional epithelium (a). QUE treated group depicting transitional epithelium within normal limits (b). DOX treated group is not illustrating any effect, very mild damage is observed which is considered not toxic (c). DOX+QUE treated group demonstrate normal morphology (d)