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ORIGINAL ARTICLE

Protective effects of *Bombyx mori*, quercetin and benazepril against doxorubicin induced cardiotoxicity and nephrotoxicity

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Abstract The present study was conducted with the aim of evaluating the protective effects of *Bombyx mori*, quercetin and benazepril on doxorubicin (DXR) induced cardiotoxicity and nephrotoxicity in rats. *B. mori*, quercetin and benazepril were administered for 7 days, and a single intravenous injection of 10 mg/kg body weight of DXR on day five. The animals were sacrificed 48 h after DXR administration. DXR produced a significant elevation in the malondialdehyde (MDA) level and significantly inhibited the activity of glutathione (GSH) in the heart and the kidney followed by the activity of catalase (CAT) in the heart tissue with a significant rise in the serum levels of aspartate transaminase (AST), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine and a reduction in serum GSH levels indicating acute cardiac toxicity. *B. mori*, quercetin and benazepril pretreatment significantly reduced the MDA concentration and ameliorated the inhibition of cardiac GSH and CAT activity. *B. mori*, quercetin and benazepril also significantly improved the serum levels of AST, LDH, BUN, creatinine and GSH in DXR-treated rats. Furthermore, histological examination of the heart sections confirmed the myocardial injury with DXR administration, and the near normal pattern with *B. mori*, quercetin and benazepril pretreatment. The results provide clear evidence that the *B. mori*, quercetin and benazepril pretreatments offer significant protection against DXR-induced enzymatic changes in serum, cardiac and renal tissue damage.

1. Introduction

Doxorubicin (DXR) is an anthracycline, glycoside antineoplastic agent which is most widely used in the chemotherapy of cancer. It has been used successfully to produce improvement in many neoplastic conditions like acute lymphoblastic

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and acute myeloblastic leukemias (Bernard, 1967; Cortex et al., 1972). However, the clinical use of this drug is limited by its side effects which are cardiomyopathy and nephropathy. The exact mechanism of doxorubicin-induced toxicity remains unclear, but most studies support the view that oxidative stress plays an important role in the pathogenesis of DXR toxicity (Kumar et al., 2001).

Bombyx mori (called silk *cocoon* or ''Abresham'' in the Unani system), is a major constituent of ''Khamira Abresham Hakim Arshad Wala'' a common traditional Unani medicine (Goyal et al., 2010). Kurioka et al. isolated three quercetin glycosides, quercetin 5-O-beta-D-glucoside, quercetin 7-O-beta-D-glucoside, and quercetin 4′-O-beta-D-glucoside, and two kaempferol glycosides, kaempferol 5-O-beta-D-glucoside and kaempferol 7-O-beta-D-glucoside, from an ethanolic extract of yellow cocoon shell of the silkworm. It was suggested that potent antioxidative activity in the cocoon is mainly due to flavonoid compounds since free radical scavenging activity was found in the cocoon flavonoids (Kurioka and Yamazaki, 2002).

Quercetin is one of the most common food flavonoid. The flavonoids frequently occur as glycosides (De Silva et al., 1998). Quercetin is able to suppress free radical processes at three stages; the formation of superoxide ion, the generation of hydroxyl (or cryptohydroxyl) radicals in the Fenton reaction and the formation of lipid peroxy radicals (Hansaki et al., 1994).

Benazepril is a non-sulfydryl, long acting Angiotensin converting enzyme inhibitor prodrug, which is converted in vitro into its active diacid form, benazeprilat. Benazepril is a safe and effective antihypertensive agent that does not cause any adverse renal or metabolic effects (Tramonti et al., 1996). Recently, benazepril has shown protective effects against chronic renal fibrosis and renal glomerulosclerosis (Chen et al., 2007).

The effectiveness of *B. mori,* quercetin and benazepril against DXR-induced cardiotoxicity and nephrotoxicity as well as oxidative stress related parameters has not been scientifically evaluated as yet. Therefore, the aim of the present study was to investigate the possible effect of *B. mori*, benazepril and quercetin against DXR-induced cardiotoxicity and nephrotoxicity in rats using biochemical markers of oxidative stress and cellular damage.

2. Materials and methods

2.1. Experimental animals

Laboratory Wistar albino rats (either sex, 10–12 week old), with body weight of 180–200 g, were used. Animals were acclimatized and housed under controlled conditions of illumination (12 h light/12 h darkness) and temperature of 20–25 °C. Standard pellet diet (Amrut Rat Feed, Mfd. By Nav Maharashtra, Ltd., New Delhi) and water *ad libitum* were provided to the animals throughout the study period. The study was approved by the Institutional Animal Ethics Committee of Jamia Hamdard, New Delhi.

2.2. Drugs and chemicals

DXR, *B. mori*, benazepril and quercetin were obtained from Dabur Research Foundation (India), Hamdard Wakf Labs, Delhi; Novartis India Ltd, and RRL, Jammu, respectively. AST, LDH, BUN and creatinine assay kits were purchased from Span Diagnostics Ltd. and J. Mitra & Co. Ltd. Delhi. All the other chemicals used were of analytical grade.

2.3. Experimental groups

After acclimatization rats were randomly assigned into eight groups of six animals each.

Group 1 received normal saline (1 ml/kg body weight), orally daily for 7 days and served as the control group.

Group 2 received a single dose of DXR (10 mg/kg i.v.), on day five, 2 hours after saline administration, served as the toxic control group.

Group 3 received *B. mori* (3 ml/kg per day p.o. for 7 days) and DXR (10 mg/kg i.v. on 5th day).

Group 4 received benazepril (10 mg/kg per day p.o. for 7 days) and DXR (10 mg/kg i.v. on 5th day).

Group 5 received quercetin (2 mg/kg per day p.o. for 7 days) and DXR (10 mg/kg i.v. on 5th day).

Group 6 received *B. mori per se* (3 ml/kg per day p.o. for 7 days).

Group 7 received benazepril *per se* (10 mg/kg per day p.o. for 7 days).

Group 8 received quercetin *per se* (2 mg/kg per day p.o. for 7 days).

Rats were sacrificed under ether anesthesia 48 h after DXR administration. Ether had no effect on the activity of the drugs and animals were sacrificed immediately afterward. Blood samples were collected from carotid artery and sera were separated by centrifugation at 3000 rpm for 10 min and frozen at −20 °C for estimation of serum activities of AST, LDH, BUN, creatinine and GSH. The heart and the kidney were isolated, weighed, washed in ice-cold physiological saline and homogenized for biochemical and histopathological studies.

2.4. Biochemical estimations

Serum AST (Reitman and Frankel, 1957), LDH (Lum and Gambino, 1974), potassium (Ahmad et al., 2002), creatinine (Bones and Tausky, 1945), blood GSH (Beutler et al., 1963), urea (Teitz, 1976) and tissue TBARS (Ohkawa et al., 1979), GSH (Sedlack and Lindsay, 1968), and catalase (Luck, 1971) were estimated according to the reported procedures.

2.5. Histopathological examination of heart sections

Formalin fixed heart sections were embedded in paraffin wax, serially sectioned (3–5 lm), and stained with Hematoxylin and Eosin, for assessment of histopathological changes (Belur et al., 1990).

3. Statistical analysis

Statistical analysis was carried out using Graph pad prism 3.0 (Graph pad software San Diego, CA). All results are expressed as mean ± S.E.M. Groups of data were compared with the analysis of variance (ANOVA) followed by Dunnett's *t*-test to identify significance among groups. Values were considered statistically significant when $p < 0.05$.

4. Results and discussion

Doxorubicin induced cardiotoxicity and nephrotoxicity are very well documented phenomenon in humans (Lefrak et al., 1973) as well as in different animal models (Singal et al., 1987; Siveski-Iliskovic et al., 1995). The mechanism of DXR-induced toxicity is not fully understood. One hypothesis proposed for this mechanism is the involvement of free radicals (Myers, 1982). This has been shown to occur through the metabolic breakdown of DXR to a free radical state that interacts with molecular oxygen to generate peroxides (Singal et al., 1987). These highly toxic reactive oxygen species react with cellular molecules including nucleic acids, protein and lipids, causing cell damage (Yin et al., 1998). Administration of DXR in combination with agents that would block its free-radical-mediated toxicity without affecting its anti-tumor activity, and prevent its oxidative stress and tissue injury, might serve as a novel combination.

In our investigation, we focused on studying the changes in cardiac enzyme activity, hypertrophy of residual myocytes and antioxidant activity. The results of this study have established that a single dose of DXR (10 mg/kg, i.v.) induces acute cardiotoxicity and nephrotoxicity in rats which are in agreement with previous studies (Pinna et al., 1994; Dziegel et al., 2002). The results of this study also showed an obvious cardiac protection by prophylactic administration of *B. mori*, benazepril and quercetin for 7 days before DXR treatment, as evidenced by biochemical and histopathological changes in the heart and the kidney. In recent studies ''Khamira Abresham Hakim Arshad Wala'', has shown protective effects in isoproterenol-induced myocardial necrosis (Goyal et al., 2010) and Alzheimer's disease (Khan et al., 2006). *B. mori* is the major constituent in this formulation.

Increased activity of serum AST and LDH is a well-known diagnostic marker of myocardial function. It has been reported that AST and LDH are released from the heart into blood stream, increasing the concentration in serum (Deepa and Varalakhshmi, 2003). In the present study, a marked elevation ($P < 0.01$) in the activities of AST and LDH in the serum of DXR-intoxicated rats was observed. Pretreatment with *B. mori*, benazepril and quercetin resulted in significant reduction ($P < 0.05$) in the activities of AST and LDH toward near normal as compared with toxic control rats. This result could be due to protective effects of *Bombyx mori*, benazepril and quercetin on the myocardium, thus restricting the leakage of AST and LDH (Table 1).

Table 2 Effect of DXR, *Bombyx mori*, quercetin and benazepril on serum BUN and creatinine levels.

Treatment regimen	Urea (mmols/L)	Creatinine (μmols/L)
Control	8.93 ± 0.91	57.33 ± 7.51
DXR	19.80 ± 1.1**	213.58 ± 7.98**
Bombyx mori + DXR	10.62 ± 0.43**	129.24 ± 12.36**
Quercetin + DXR	13.87 ± 1.1**	151.70 ± 10.25*
Benazepril + DXR	12.64 ± 0.69**	139.87 ± 5.37**
Bombyx mori per se	9.85 ± 0.23	70.72 ± 12.23
Quercetin *per se*	11.14 ± 0.43	65.33 ± 10.65
Benazepril *per se*	9.29 ± 0.88	71.45 ± 3.05

DXR (toxic control) is compared with the normal control. *Bombyx mori*, benazepril and quercetin are compared with the toxic control.
* $P < 0.05$.
** $P < 0.01$.

Potassium (normal values: 3.5–5.0 mmoles/L) is a major component in cardiac function. Elevated potassium level, called hyperkalemia occurring due to decrease in renal function, is the most likely cause and others are major infection, gastrointestinal bleeding, and rapid protein breakdown which may also cause elevated potassium levels. Electrocardiogram changes can be seen in this condition. In our study, we found an increase ($P < 0.01$) in serum K^+ levels in DXR-treated rats that reveals the adverse effect of DXR on cardiac and renal cells. The significant prevention ($P < 0.01$) of DXR-induced changes in K^+ level by *B. mori*, benazepril and quercetin showed the prominent protective effect on cardiac and renal cells (Table 1).

Furthermore, we observed an increase ($P < 0.01$) in the levels of nephrocyte injury markers like urea and creatinine in the serum of DXR-treated rats indicating the nephrotoxic effect of DXR in rats. The attenuation ($P < 0.01$) of DXR-induced rise in serum urea and creatinine levels by *B. mori*, benazepril and quercetin indicates the reno-protective effect of these drugs (Table 2).

GSH functions as a free radical scavenger in the repair of radical induced cellular damage. Others also have reported low levels of GSH as observed during an increase in oxidative stress caused by doxorubicin administration (Singal and Iliskovic, 1998). The attenuation ($P < 0.01$) of glutathione depleting effects of DXR by *B. mori*, benazepril and quercetin in blood, heart and kidney tissues reveals the antioxidant effects produced by these drugs (Figs. 1 and 2).

Table 1 Effect of DXR, *Bombyx mori*, quercetin and benazepril on serum AST, LDH, GSH and K^+ levels.

Treatment regimen	AST (μkat/L)	LDH (U/L)	GSH (mg%)	K^+ (mmols/L)
Control	0.852 ± 0.079	70 ± 2.51	7.46 ± 1.44	4.0 ± 0.31
DXR	5.142 ± 0.147**	724 ± 10.16**	3.46 ± 0.17*	8.18 ± 0.76**
Bombyx mori + DXR	1.871 ± 0.06**	393 ± 8.8**	11.76 ± 0.98**	4.86 ± 0.23**
Quercetin + DXR	2.82 ± 0.39*	487 ± 45.6*	13.81 ± 0.88**	3.86 ± 0.1**
Benazepril + DXR	2.34 ± 0.14*	352 ± 8.1**	10.76 ± 1.32**	4.7 ± 0.28**
Bombyx mori per se	1.08 ± 0.15	96 ± 8.79	8.79 ± 0.43	3.28 ± 0.42
Quercetin *per se*	0.974 ± 0.18	103 ± 13.2	10.44 ± 1.04	4.1 ± 0.21
Benazepril *per se*	0.865 ± 0.28	94.9 ± 11.1	8.94 ± 0.21	4.26 ± 0.27

DXR (toxic control) is compared with the normal control. *Bombyx mori*, benazepril and quercetin are compared with the toxic control.
* $P < 0.05$.
** $P < 0.01$.

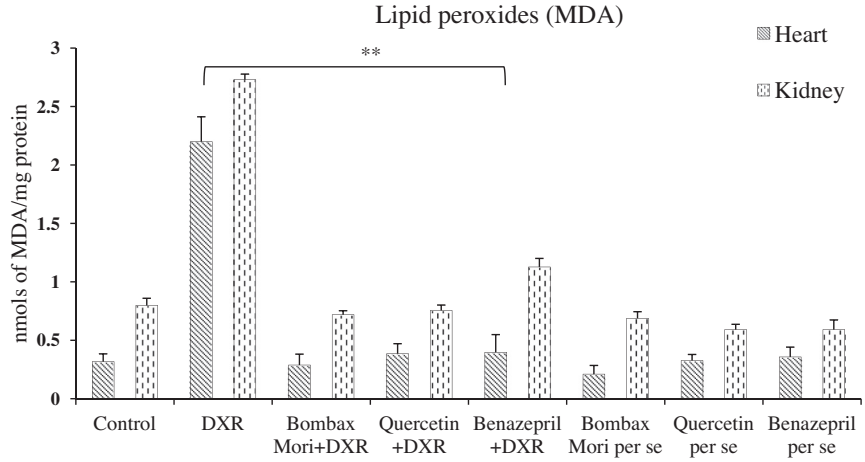

Figure 1 Effect of DXR, *Bombyx mori*, quercetin and benazepril on the heart and kidney MDA level. DXR (toxic control) is compared with the normal control. *Bombyx mori*, benazepril and quercetin are compared with the toxic control. Values are statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$.

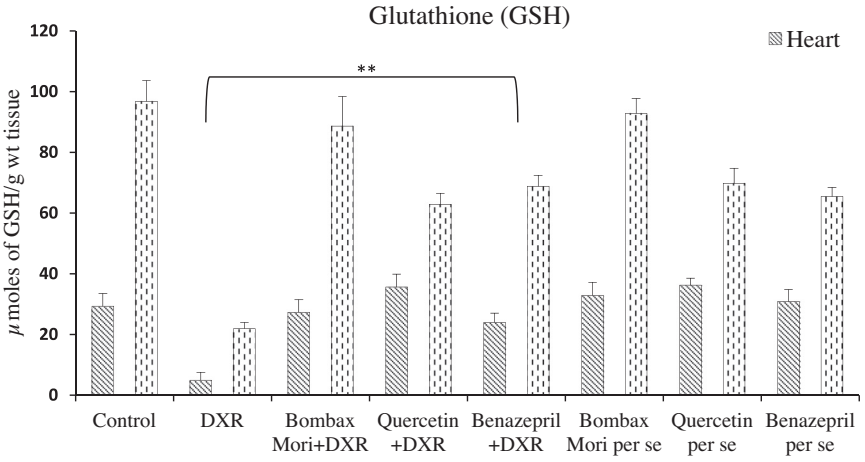

Figure 2 Effect of DXR, *Bombyx mori*, quercetin and benazepril on the heart and kidney GSH level. DXR (toxic control) is compared with the normal control. *Bombyx mori*, benazepril and quercetin are compared with the toxic control. Values are statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$.

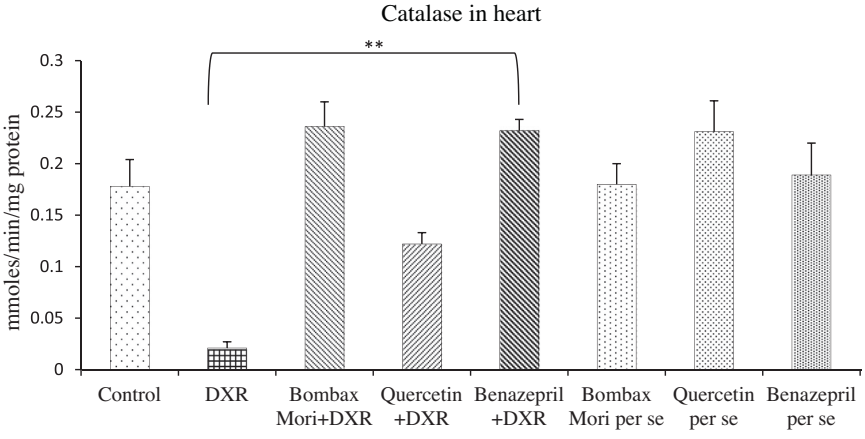

Figure 3 Effect of DXR, *Bombyx mori*, quercetin and benazepril on heart catalase activity. DXR (toxic control) is compared with the normal control. *Bombyx mori*, benazepril and quercetin are compared with the toxic control. Values are statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$.

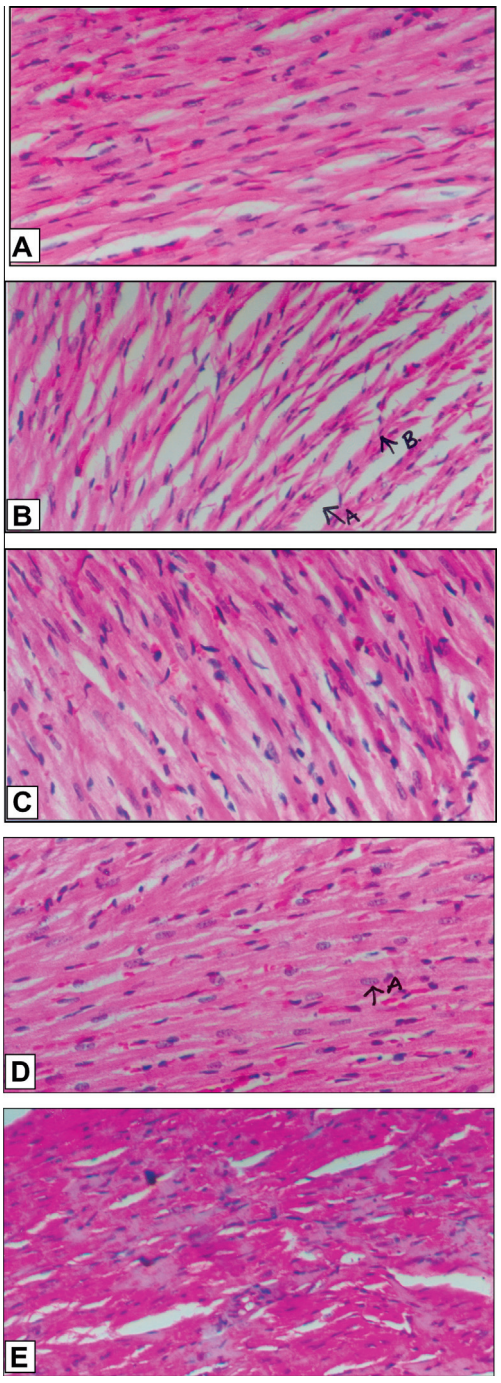

Figure 4 (A–E) Histopathological slides of rat heart (H&E × 100). (A) Control rat heart shows normal cellular morphology. (B) DXR treated rat heart shows a small cluster of myocardial fibers, widening of myofibrils (indicated by arrow A), loss of myofibril integration and cell shrinkage (indicated by arrow B). (C) *Bombyx mori* + DXR treated rat heart showing cardiac muscle fiber of normal shape size and configuration. (d) Quercetin + DXR treated rat heart showing normal myocardium. No vacuolated fibers are observed. (E) Benazepril + DXR treated rat heart showing normal cardiac muscle fiber but at some places hyalinaization of myofibres are observed.

B. mori cocoon is reported to contain flavonoids (Kurioka and Yamazaki, 2002; Hirayama et al., 2006). It is suggested that potent antioxidative activity in the cocoon is mainly due to flavonoid compounds since free radical scavenging activity was found in the cocoon flavonoids (Kurioka and Yamazaki, 2002). Thus, the protective effect produced by *B. mori* and quercetin might be due to their antioxidant property.

DXR can undergo a two-electron reduction to form alkylating quinone and methides. As quinones, they are also able to undergo redox cycling to generate oxygen radicals, which can in turn lead to the induction of lipid peroxidation. Increased levels of oxygen species due to doxorubicin have been detected by an increase in tissue malondialdehyde (MDA) formation, which is a breakdown product of lipid peroxidation (Alafeefy, 2011; Myers et al., 1977). Significant elevation ($P < 0.01$) in the level of MDA after DXR administration was observed in our study. The reduction ($P < 0.01$) in levels of MDA in heart and kidney tissues of rats pretreated with *B. mori*, benazepril and quercetin in DXR-treated rats suggests the anti-lipid peroxidative property of test drug that protects the myocardium from lipid peroxidation (Figs. 1 and 2).

CAT is an endogenous antioxidant enzyme present in the cytoplasm that detoxifies the free radical hydrogen peroxide into water and oxygen molecules. The reduced ($P < 0.01$) CAT activity in the heart of rats treated with DXR indicates depletion of this antioxidant enzyme which is in agreement with previous studies (Rajaprabhu et al., 2007; Arafa et al., 2005). Pretreatment with *B. mori*, benazepril and quercetin resulted in restoration ($P < 0.01$) of CAT activity which reflects the possible protective effect of *B. mori*, benazepril and quercetin (Fig. 3).

Furthermore, DXR-induced cardiotoxicity is manifested by altered histopathological features including focal myocardial fibrosis and disorganization of the myocardium of myofibrillar loss (Saad et al., 2001; Yilmaz et al., 2006). In our study the Toxic control group showed a small cluster of myocardial fibers, widening of myofibrils, loss of myofibril integration and cell shrinkage (Fig. 4B). The samples from the groups pretreated with *B. mori* (Fig. 4C), benazepril (Fig. 4D) and quercetin (Fig. 4E) before administration of DXR all showed an improved myocardium and no evidence of vacuolar myopathy except benazepril that showed hyalinization of myofibrils in some places. A normal histological appearance of the myocardium was also observed in the normal control group. Thus, histopathological results also indicate a protective effect of *B. mori* against DXR-induced cardiotoxicity in rats.

5. Conclusion

In conclusion, the present findings indicate that *Bombyx mori*, benazepril and quercetin have a protective effect against the acute model of DXR-induced cardiotoxicity and nephrotoxicity in rats.

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