The protective effect of a purified extract of *Withania somnifera* against doxorubicin-induced cardiac toxicity in rats

A. Hamza · A. Amin · S. Daoud

Abstract The therapeutic value of doxorubicin as an effective antineoplastic agent is limited by its cardiotoxic side-effects. The administration of doxorubicin (10 mg/kg) to male Wistar rats induced necrosis and apoptosis in heart tissues. It also caused oxidative stress damage as evidenced by the elevation of malondialdehyde and protein carbonyl levels and catalase activity, accompanied by the concurrent depletion of total antioxidant capacity and of superoxide dismutase level in cardiac tissues. The doxorubicin-induced cardiotoxicity and oxidative stress damage were also accompanied by increases of myeloperoxidase activity, total calcium content, and the expression of Bcl-2 protein in heart tissues. Most of these doxorubicin-induced biochemical and histological alterations were effectively attenuated by prior administration of purified standardized extract (1.5% withanolides; manufactured by Idea Sphere Inc., American Fork, UT, USA) of *Withania somnifera* (300 mg/kg). Thus, *Withania* may play a role in the protection against cardiotoxicity and thus might be a useful adjuvant therapy where doxorubicin is the cancer-treating drug.

Abbreviations

AST	aspartate aminotransferase
CAT	Catalase
DXR	Doxorubicin
FRAP	ferric reducing antioxidant power
LP	lipid peroxidation
MDA	Malondialdehyde
MPO	Myeloperoxidase
ROS	reactive oxygen species
SOD	superoxide dismutase
TAC	total antioxidant capacity
TBA	thiobarbituric acid
TUNEL	terminal deoxynucleotidyl transferase mediated dUTP nick end labelling
WIT	*Withania somnifera* Dunal

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Introduction

Doxorubicin (DXR), an anthracycline antibiotic, is a commonly used antineoplastic agent for many types

of cancers including lymphoma, leukemia, and solid tumors. However, DXR is known to be associated with various toxic effects such as cardiotoxicity (Yagmurca et al. 2003; Fujimura et al. 2004; Oliveira et al. 2004; Yilmaz et al. 2006). DXR-induced cardiotoxicity is mediated by the formation of free radicals that oxidatively damage many cellular components (Mukherjee et al. 2003; Yagmurca et al. 2003; Oliveira et al. 2004). DXR is enzymatically reduced to its semiquinone radical (Minotti et al. 2001). This DXR semiquinone radical directly transfers its electron to molecular oxygen, generating a superoxide radical and hydrogen peroxide (Gille and Nohl 1997). As reactive oxygen species (ROS) play important roles in DXR-induced toxicity, many compounds with antioxidant properties have been examined as potential therapeutic and/or protective agents. Among these compounds were *p*-coumaric acid (Abdel-Wahab et al. 2003), garlic acid (Mukherjee et al. 2003), erdosteine (Yagmurca et al. 2003), carvedilol (Oliveira et al. 2004), and lycopene (Yilmaz et al. 2006).

Withania somnifera Dunal (WIT), family *Solanaceae*, is an annual herb that is most commonly known as ashwagandha. WIT has been used extensively as a valuable drug in Ayurveda, the Indian traditional system of medicine (Mishra et al. 2000; Scartezzini and Speroni 2000). Withanolides are the major active constituents of WIT that are isolated from its root and leaves (Mishra et al. 2000; Scartezzini and Speroni 2000). Previous studies have shown that WIT possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, and hepatoprotective properties (Mishra et al. 2000; Scartezzini and Speroni 2000). Cardioprotective effects of WIT have also been documented against myocardial damage induced by strophanthin-K (Dhuley 2000) and by isoproterenol (Mohanty et al. 2004) in different animal models. Moreover, administration of WIT extract was found to reduce the myelosuppression (Davis and Kuttan 1998) and urotoxicity (Davis and Kuttan 2000) induced by antineoplastic agents such as cyclophosphamide in mice. WIT roots have also shown a specific chemopreventive efficacy against forestomach and skin carcinogenesis in mice (Padmavathi et al. 2005).

The purpose of this investigation was to evaluate the protective effects of WIT against DXR-induced cardiotoxicity in male rats and to study the mechanism underlying these effects.

Materials and methods

Chemicals

The herbal formulation of WIT tablets (Ashwaganda; 205154311) was manufactured by Idea Sphere Inc., American Fork, UT, 84003 USA. The herbal formulation of root and leaf tablets contains 300 mg extract, which is standardized by the manufacture company to 1.5% withanolides (4.5 mg of withanolides in each tablet). Apoptag plus Peroxidase in situ Apoptosis Detection Kit was purchased from Chemicon International Inc., Temecula, CA, U.S.A. A kit for serum aspartate aminotransferase activity was purchased from BioMerieux, RCS Lyon, France. *o*-Dianisidine, 2,4-dinitrophenylhydrazine, thiobarbituric acid, Folin reagent, epinephrine, SOD enzyme, H_2O_2 and bovine albumin were obtained from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were obtained from local commercial suppliers.

Animals

Adult male albino rats, Wistar strain (150–200 g), were obtained from the Animal House, UAE University, U.A.E. Rats were maintained on standard pellet diet and tap water *ad libitum* and were kept in polycarbonate cages with wood chip bedding under a 12 h light/dark cycle and at room temperature 22–24°C. Rats were acclimatized to the environment for one week prior to experimental use. The study was approved by the Animal Research Ethics Committee, UAE University.
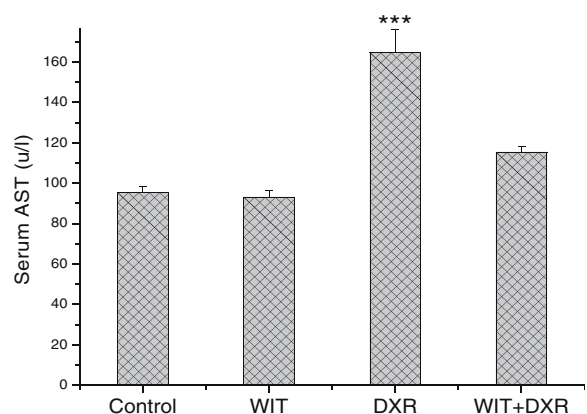
Fig. 1 Effect of WIT on serum AST activity in control and DXR-treated rats. Each column represents the mean ± SEM ($n=6$). ***$p<0.001$ vs. control

Fig. 2 Photomicrograph of the heart tissues of control (a) and WIT-treated rats (b) showing the normal myofibrillar structure with striations and branched appearance. Heart tissues of DXR-treated rats (c, d) showing focal necrosis of muscle fibers with eosinophilia in the cytoplasm (arrows). Heart tissues of DXR-treated rats also show deformations of muscle fibers with focal hemorrhage (arrowheads) and inflammatory cell infiltrations (stars). Rats treated with WIT+DXR (e, f) before DXR, exhibiting relatively normal myocardial cells with vascular dilatation and moderate degeneration of some fibril cells. (hematoxylin and eosin, ×200.)
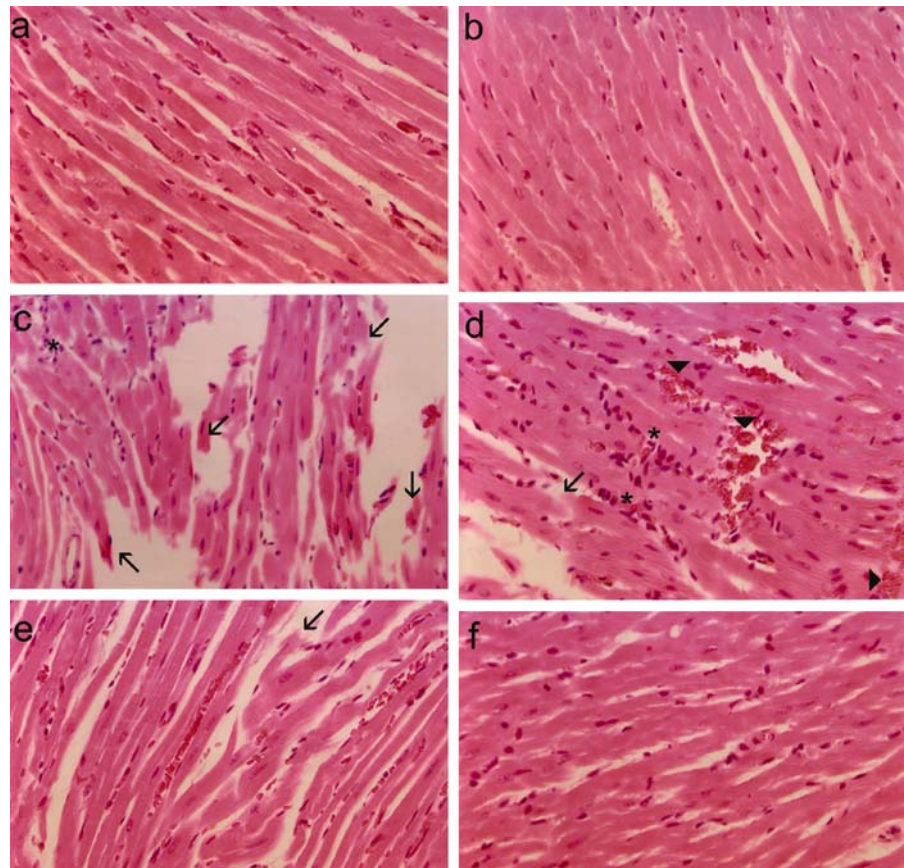

Treatment regime

DXR (Adriamycin) solution was freshly prepared in a saline solution. WIT tablets were suspended in distilled water. This extract was then used at a dose of 300 mg/kg body weight (bw) (4.5 mg of withanolides), which is equivalent to a volume of 5 ml/kg bw. Rats were randomly divided into four groups ($n=6$) and were subjected to the following treatments. In the DXR-treated group, animals were orally administered a dose of distilled water (5 ml/kg bw) for 14 days. After the first 7 days, animals of this group received a single intraperitoneal dose of DXR (10 mg/kg bw) DXR was freshly prepared in a saline solution. This DXR dose was selected as it has been used previously to induce cardiotoxicity in male albino rats (Jang et al. 2004; Yilmaz et al. 2006). Animals of the protective group were fed WIT extract for 14 days and, similarly, cardiotoxicity was induced by injecting the same single dose of DXR after 7 days of WIT administration. The third group was treated with WIT daily for 14 days and

injected with single dose of saline (5 ml/kg bw) after 7 days of WIT treatment. The control group was treated with distilled water (5 ml/kg bw) for 14 days and injected with single dose of saline after 7 days of water administration. At the end of treatments and after the last WIT or vehicle solution administration, blood and heart tissues were collected from all groups and stored at −20°C for further processing.

Sample preparation

Following diethyl ether anesthesia, blood was collected from the retro-orbital plexus. Diethyl ether-anesthetized rats were then sacrificed and heart tissues were removed. For histopathological examination, the left half of the heart was immediately fixed in 10% buffered formalin. For biochemical determination, the right half of the heart was homogenized in ice-cold KCl (150 mmol/L). The ratio of tissue weight to homogenization buffer was 1:10. From the latter, suitable dilutions were prepared to determine the

Fig. 3 TUNEL-positive cells in the heart tissues of rats treated with vehicle (a, control), WIT (b), DXR (c), and WIT+DXR (d). Photomicrographs (a–d) and the semi-quantitative analysis (e) show variable levels of apoptosis in different experimental groups. The number of apoptotic cells in each section was calculated by counting the number of TUNEL-positive cells in 10 fields at 400× magnification. Brown staining indicates TUNEL-positive cells. TUNEL-positive cells are indicated by arrows (hematoxylin counterstained, 400×). ***$p<0.001$ vs. control
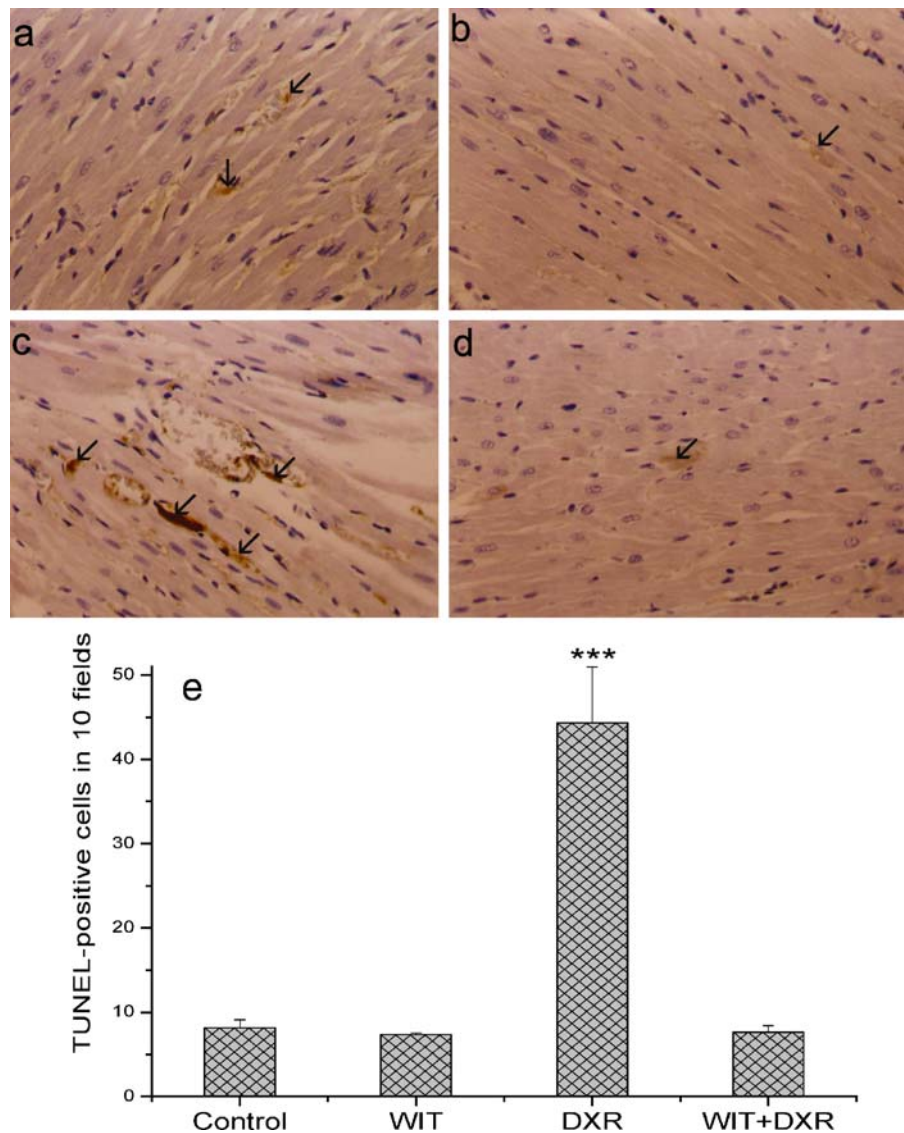
levels of calcium, protein carbonyl (P.carbonyl), malondialdehyde (MDA), total antioxidants, and total proteins. To assess the activity of superoxide dismutase (SOD), catalase (CAT), and myeloperoxidase (MPO), suitable dilutions were made in different buffers. To obtain serum, blood was collected in centrifuge tubes and centrifuged in a refrigerated centrifuge (4°C) at 1300 g for 20 min.

Biochemistry and histopathology

MDA is the most abundant individual aldehyde resulting from lipid peroxidation (LP) breakdown in biolog-ical systems and is commonly used as an indirect index of LP (Sorg 2004). Determination of MDA, as described by Uchiyama and Mihara (1978), is based on its reaction with thiobarbituric acid (TBA) to form a pink complex with absorption maximum at 535 nm.

CAT activity was determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed in units/mg of protein as described by Aebi (1984).

MPO activity in cardiac homogenate was determined as described in Hillegas et al. (1990). The substrate for MPO assay was potassium phosphate buffer (pH 6.0) containing 0.53 mmol/L o-dianisidine and 20 mmol/L H_2O_2. Absorbance of the sample was
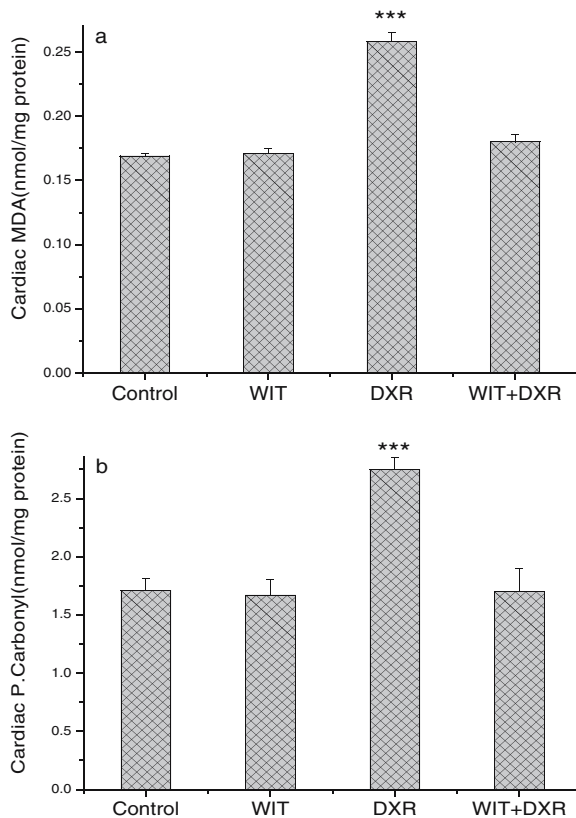
Fig. 4 Effect of WIT on MDA level (a) and P.carbonyl content (b) in heart tissues of control and DXR-treated rats. Each column represents the mean ± SEM ($n=6$). ***$p<0.001$ vs. control

monitored at 460 nm over a period of 3 min. One unit of MPO was defined as the amount of MPO that degrades 1 μmole peroxide per minute.

In heart tissues, the SOD enzyme activity was determined according to the method described by Sun and Zigman (1978). This method is based on the ability of SOD to inhibit the auto-oxidation of epinephrine at alkaline pH to adrenochrome and other derivatives, which are easily monitored in the near-UV region of the absorption spectrum.

P.carbonyl contents were determined according to the method of Reznick and Packer (1994). This method is based on spectrophotometric detection of the end product of the reaction of 2,4-dinitrophenylhydrazine with P.carbonyl to form protein hydrazones at 370 nm. The results were expressed as nmoles of carbonyl group per milligram of protein with molar extinction coefficient of 22 000 L $M^{-1}cm^{-1}$.

The total antioxidant capacity (TAC) in heart tissues was evaluated with the ferric reducing antioxidant power (FRAP) assay. The FRAP assay was determined according to the method described by Benzie and Strain. (1996). The FRAP assay measures the change in absorbance at 593 nm caused by the formation of a blue-colored ferrous-tripyridyltriazine complex from an initially colorless oxidized ferric form. Tissue-embedded electron-donating antioxidants drive the reduction of Fe(III) to Fe(II).

Serum aspartate aminotransferase (AST) activity was measured spectrophotometrically using a Shimadzu recording spectrophotometer (UV-160). The assay was performed using a BioMerieux reagent kit, according to the protocol supplied with the kit.

Calcium content was measured in heart tissues using inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian vista-MPXCCD Simultaneous ICP-OES). Heart samples were digested with concentrated nitric acid and perchloric acid and

Table 1 Effect of WIT on levels of antioxidants, total antioxidant capacity (FRAP) and calcium in heart tissues of control and DXR-treated rats

Parameter[a]	Treatment			
	Control	WIT	DXR	WIT+DXR
CAT	4.64±0.12	4.76±0.10	6.03±0.19***	5.10±0.05*
SOD	1.71±0.03	1.72±0.01	1.22±0.11***	1.66±0.09
FRAP	26.10±0.23	25.70±0.42	23.59±0.38***	26.47±0.24
Calcium	86.87±2.40	86.15±1.18	137.40±4.81***	100.84±16.16

Values are expressed as mean ± SEM ($n=6$).

[a] Units of FRAP and calcium are nmol/mg protein; units of SOD and CAT enzymes are units/mg protein.

*$p<0.05$; ***$p<0.001$ vs. control.
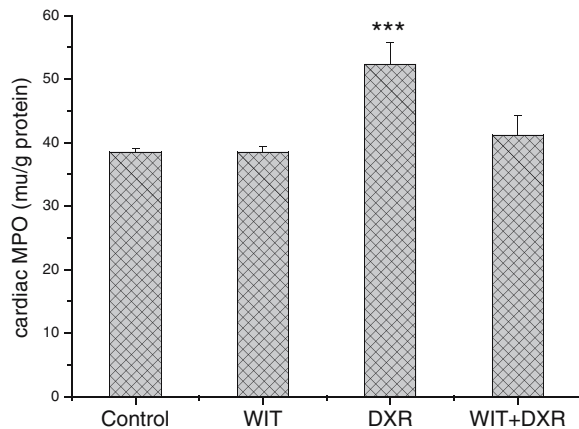
Fig. 5 Effect of WIT on MPO activity in heart tissues of control and DXR-treated rats. Each column represents the mean ± SEM ($n=6$). ***$p<0.001$ vs. control

heated up gently to 200°C until digestion was complete.

The total protein contents of heart tissues were determined according to the Lowry method as modified by Peterson (1977). Absorbance was recorded using a Shimadzu recording spectrophotometer (UV-160) for all measurements.

For the histological examinations, small pieces of heart tissue were fixed in 10% neutral phosphate-buffered formalin. Hydrated tissue sections, 5 μm in thickness, were then stained with hematoxylin and eosin. The sections were examined under a Leica DMRB/E light microscope.

In-situ localization of apoptotic cells

Apoptosis was assessed in deparaffinized sections using the TUNEL technique. In this technique, the manufacturer's protocol for the Apoptag plus Peroxidase in Situ Apoptosis Detection Kit (Chemicon International, CA, USA) was followed. This method detects the DNA fragmentation associated with apoptosis by labeling 3-OH DNA termini with digoxigenin-nucleotides, a process facilitated by terminal deoxynucleotidyl transferase. The labeled fragments are then allowed to bind to anti-digoxigenin antibody conjugated with peroxidase. Color was developed by adding sufficient peroxidase substrate to specimens. The number of apoptotic cells in each section was calculated by counting the number of TUNEL-positive apoptotic cells in 10 fields per slide at 400× magnification.

Immunohistochemical analysis of Bcl-2

The expression of Bcl-2 protein was studied on sections by immunohistochemistry. Briefly, after deparaffinization and rehydration, tissue sections were treated with 3% hydrogen peroxide for 20 min to diminish nonspecific staining. The sections were immersed in 10 mmol/L citrate buffer solution (pH 6.0) in a microwave oven twice for 5 min and then incubated with normal goat serum for 20 min. Sections were incubated for overnight at 4°C with the rabbit Bcl-2 primary antibody and were then washed with phosphate-buffered saline. Sections were exposed to the avidin-biotin-peroxidase complex (1/400, Dako) for 1 h at room temperature. The chromogenic substrate of peroxidase was developed using a 0.05% solution of 3,3-diaminobenzidine tetrahydrochloride, 0.03% hydrogen peroxide, and imidazol in Tris-HCl buffer (pH 7.6). Sections were counterstained with hematoxylin. The number of immunostained cells was counted in each section per 10 microscopic fields at 400× magnification.

Statistical analysis

SPSS (version 10) (SPSS Inc., Chicago, IL, USA) was used to carry out a one-way analysis of variance (ANOVA) on our data. When significant differences were detected by ANOVA, analyses of differences between the means of the treated and control groups were performed using Dunnett's t-test.

Results

Biochemical and histological effects on heart tissues

The levels of serum AST as a marker of heart damage significantly increased ($p<0.001$) compared to the control after treatment with DXR. The treatment with WIT alone did not show any significant changes in the AST activity (Fig. 1), while concomitant treatment with WIT and DXR treatment attenuated this increase in AST level.

Figure 2a shows the normal architecture of control cardiac muscles. Severe degenerations of the myofibrils, with focal necrosis, and vacuolated cytoplasm were clearly seen in the DXR-treated group. DXR intoxication also induced eosinophilic cytoplasm and focal

Fig. 6 The protein expression of Bcl-2 in the heart tissues of rats treated with vehicle (a, control), WIT (b), DXR (c), and WIT +DXR (d). Photomicrographs (a–d) and the semi-quantitative analysis (e) show the degree of immunostained cells in different experimental groups. The number of Bcl-2-positive cells in each section was calculated by counting the number of immunostained cells in 10 fields at 400× magnification. Brown staining indicates positive-stained cells. Bcl-2-positive cells are indicated by arrows (hematoxylin counterstain, 400×). ***$p<0.001$ vs. control
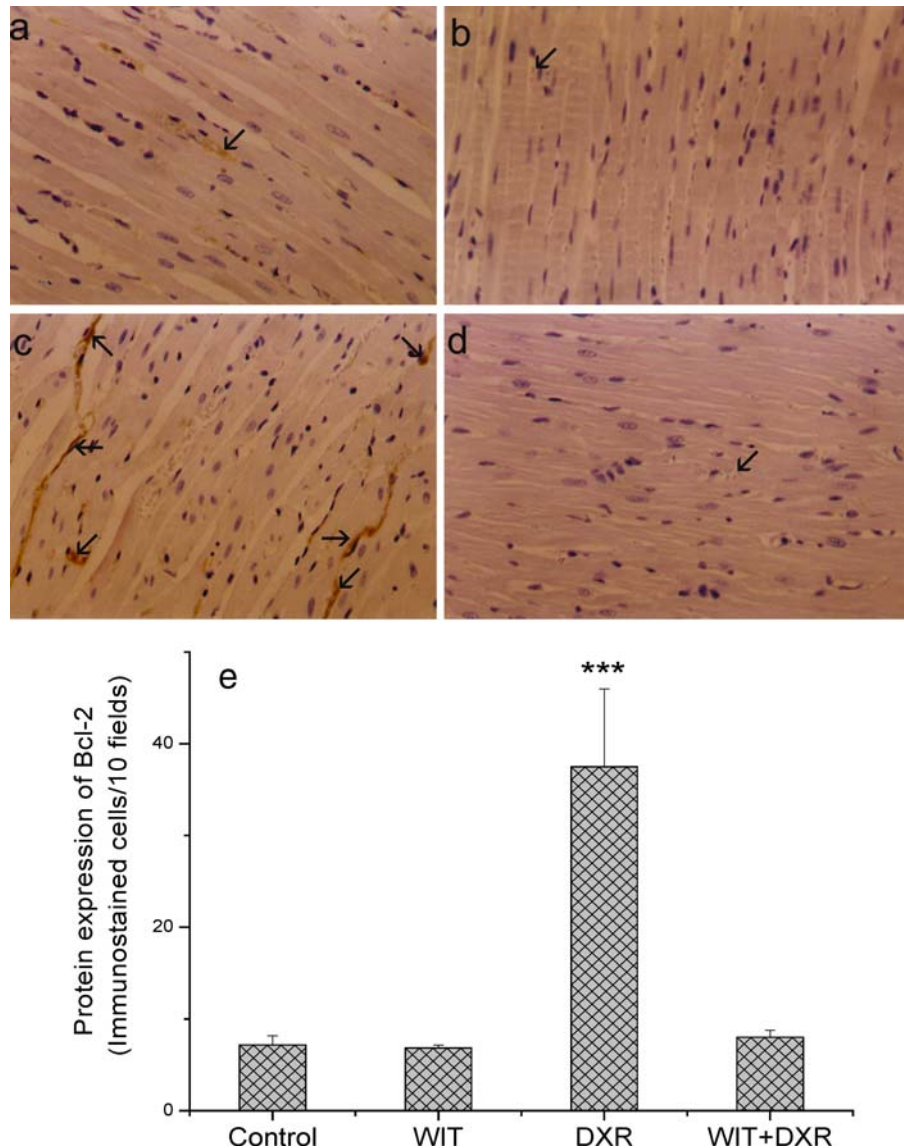
hemorrhage and with inflammatory cell infiltrations (Fig. 2c,d). Animals pretreated with WIT showed better-preserved appearance of cardiac muscle fibers with slight degeneration and some leukocyte infiltration (Fig. 2e,f). Heart tissues of WIT-treated rats shows normal myofibrillar structure (Fig. 2b).

Cell death

TUNEL assay was used to identify apoptotic cells in the heart tissues. Brown staining, indicating TUNEL-positive nuclei, was visible in heart tissues of control and WIT-treated animals (Fig. 3a,b). TUNEL-positive cells were significantly increased ($p<0.001$), however, in the DXR-treated group compared to the control group (Fig. 3c,e). Concomitant treatment with WIT and DXR significantly prevented the increase in the number of TUNEL-positive cells in DXR treated group (Fig. 3d,e).

Effects on MDA and P.carbonyl levels

Levels of both MDA and P.carbonyl were significantly elevated ($p<0.001$) in heart tissues of DXR-treated rats compared to control values (Fig. 4a,b). Concur-

rent treatment with WIT and DXR prevented the elevations of these oxidative stress markers. WIT alone, however, exhibited no significant effects on the MDA and P.carbonyl levels when compared to control groups.

Changes of antioxidants

Heart tissues of rats treated with DXR showed significant depletion in SOD activity ($p<0.001$) and in TAC (FRAP) content ($p<0.001$). In contrast, the activity of CAT enzyme was elevated significantly ($p<0.001$) in heart tissues from this group of rats (Table 1). Interestingly, the treatment with WIT prevented the depletions in SOD activity and TAC content as well as attenuating the elevation in CAT activity. Treatment with WIT alone exhibited no significant effect on the enzyme activity of SOD and CAT or on the content of TAC, compared with control group.

Change in MPO enzyme activity

WIT treatment alone did not induce any change in the activity of MPO enzyme compared to the control group. In contrast, the DXR-treated group exhibited significant ($p<0.001$) elevation in the MPO activity in the heart tissues, compared to the control group (Fig. 5). No significant effect on the MPO enzyme activity was detected between the WIT+DXR and control groups.

Effects on cardiac calcium concentrations

DXR-treated rats showed a significant increase ($p<0.001$) in heart-specific calcium content (Table 1) in heart tissues. The calcium concentration did not differ significantly from control levels when WIT was administered before DXR treatment. At the same time, WIT alone exhibited no significant effect on calcium levels in heart tissues compared to the control group.

Effects on Bcl-2 protein expression

Using immunohistochemistry, Bcl-2 protein expression was detected in hearts of control and WIT-treated animals (Fig. 6a,b). Brown staining, indicative of positively immunostained cells, was significantly ($p<0.001$) increased in the DXR-treated group compared to the

control group (Fig. 6c,e). Concomitant treatment with WIT and DXR significantly prevented the increase in the number of Bcl-2-positive cells in the DXR-treated group (Fig. 6d,e).

Discussion

This paper reports an investigation of the protective effects of a standardized extract of WIT against cardiotoxicity and DXR-induced oxidative stress in an animal model. DXR has been reported to cause cardiomyopathy in various animal species (Yagmurca et al. 2003; Fujimura et al. 2004; Oliveira et al. 2004, Yilmaz et al. 2006). The present results showed a significant elevation in serum levels of AST (Fig. 1) (a reliable biomarker of cardiac injury), after treatment with DXR at dose level 10 mg/kg bw. Similarly to many other studies, the DXR-induced myocardial damage reported here was associated with clear myocardial histopathological lesions (myocyte necrosis, degeneration, and inflammatory cell infiltrations, Figs. 2 and 3) (Mukherjee et al. 2003; Yagmurca et al. 2003; Fujimura et al. 2004; Yilmaz et al. 2006).

Oxidative stress is an important biological process that is believed to cause severe damage to cell membranes (Sorg 2004). Oxidative stress has also been implicated as the major contributing factor to the DXR-induced deformation of heart tissues (Dalloz et al. 1999, Yagmurca et al. 2003; Oliveira et al. 2004). In the present work, DXR-induced oxidative stress is confirmed by the elevation of oxidized lipids (MDA) and proteins (P. carbonyl). Interestingly, the DXR-induced oxidation of lipids and proteins were prevented by WIT (Fig. 4), suggesting an antioxidant effect of this herb. In addition, DXR reduced the TAC content and the antioxidant activity of SOD enzyme in the heart (Table 1). Free radicals have been shown to exhaust the antioxidant defense system and hence elevate the oxidation process of both lipids and proteins in heart tissues of DXR-treated rats (Dalloz et al. 1999; Yagmurca et al. 2003; Oliveira et al. 2004). In that context, the anthracycline ring structure of DXR has been shown to induce both enzymatic and nonenzymatic single-electron redox cycle liberation of ROS from molecular oxygen (Gille and Nohl 1997). In the present study, the depletion in antioxidant defense mechanism was associated with the elevation of CAT activity, an

enzyme that is normally responsible for H_2O_2 elimination (Sun and Zigman 1978), in heart and other tissues. Similar results have been reported in male Wistar rats treated with the same dose of DXR as used in this work (Dalloz et al. 1999; Yilmaz et al. 2006). This explains, at least in part, the massive production of H_2O_2 and its significant role in DXR-induced cardiotoxicity. The redox-cycle of DXR in the presence of cytochrome P450 reductase and NADPH was also shown as a major source of H_2O_2 in tissues (Gille and Nohl 1997).

WIT alone had no effect on the normal levels of oxidative stress markers (MDA and P.carbonyl). The same was true for the antioxidant profile (TAC, CAT, and SOD) as treatment with WIT alone did not show any effect. However, the concomitant treatment with WIT and DXR restored normal levels of cardiac oxidative stress markers and of antioxidants. WIT extract has been reported to attenuate or inhibit both the lipid and protein oxidative damage induced by copper (Gupta et al. 2003) and by isoproterenol (Mohanty et al. 2004) in the spinal cord and the heart of animal models. It was also shown that WIT was able to scavenge the free radicals produced as a result of lead toxicity in liver and kidney of mice as evidenced by decreased lipid peroxidation and increased activity of antioxidant enzymes in tissues (CAT and SOD) (Chaurasia et al. 2000). It was argued that the antioxidant effect of WIT can be attributed to the presence of steroidal lactones, withanolides, which represent the main active component of WIT (Mishra et al. 2000).

ROS can also be produced and accumulated in the inflammatory cells within cardiac tissues. In the present study, cardiac MPO activity was increased in DXR-treated rats (Fig. 5). This is consistent with results from a previous study (Yagmurca et al. 2003) and indicates an acute inflammation and leukocyte accumulation in heart tissues of the DXR-treated animals (Nicholls and Hazen 2005). During inflammation, activated neutrophils and some polymorphonuclear leukocytes generate a variety of highly reactive oxidants such as hypochlorous acid that are capable of oxidation, halogenation, and nitration of cellular macromolecules (Klebanoff 1999; Nicholls and Hazen 2005). WIT treatment prevented the elevation in renal MPO activity, which indicates inhibition of neutrophil infiltration and inflammation. WIT was reported to possess anti-inflammatory and immunosuppressive

properties both *in vivo* and *in vitro* (Mishra et al. 2000; Rasool and Varalakshmi 2006).

In addition to their ability to directly inflict damage upon cellular macromolecules, ROS play a pivotal role in apoptosis by initiating mitochondrial damage and activating sensitive signaling pathways (Kannan and Jain 2000). It has been documented that apoptosis plays a crucial role in the pathogenesis of DXR-induced cardiac damage (Fujimura et al. 2004, Jang et al. 2004). In this study, the apoptosis was confirmed by increase in TUNEL-positive cells in heart tissues of DXR-treated rats (Fig. 6). In fact, the anticancer property of DXR has been shown to be mediated by oxidative DNA damage and apoptosis that are a direct result of the DXR-induced production of H_2O_2 (Mizutani et al. 2005). In agreement with previous investigations, the DXR-induced apoptotic effect (Fig. 6c) shown in this study was associated with an increased expression of anti-apoptotic protein Bcl-2 (Childs et al. 2002, Jang et al. 2004). Bcl-2 protein has been shown to prevent ROS-induced apoptosis through an antioxidant mechanism (Kannan and Jain 2000). Thus, the upregulation of Bcl-2 in the present study may represent an adaptive mechanism during the generation of ROS. Administration of WIT alone did not alter the basal level of apoptotic cells and apoptosis-related protein Bcl-2 (Fig. 6b). However, concomitant treatment of WIT with DXR partially attenuated the elevation of apoptosis and restored normal levels of Bcl-2, suggesting antioxidant and antiapoptotic roles of WIT (Fig. 6d).

Accumulation of intracellular calcium level may lead to both necrotic and apoptotic cell death. ROS is known to cause damage to calcium channels and therefore to cause calcium influx into the cytoplasm from internal cellular stores and from the extracellular spaces (Ermak and Davies 2001; Waring 2005). Necrotic cell death induced by calcium overload is generally thought to be due to the activation of cellular enzymes such as proteases, nucleases, and lipases (Waring 2005). In addition, increased intracellular calcium is associated with mitochondrial calcium accumulation and activation of caspases, which initiate apoptotic cell death (Kannan and Jain 2000; Waring 2005). In the present study, DXR induced an elevation in intracellular calcium concentration while concomitant treatment with WIT and DXR caused a marked reduction in calcium levels in heart tissues (Table 1). This protective effect could be attributed to

the WIT antioxidant property proposed in this study and/or its calcium antagonistic property observed in a previous study (Choudhary et al. 2005).

In conclusion, DXR induced elevation of the oxidation end products of lipids and proteins. It also increased the MPO activity and intracellular calcium. However, DXR decreased TAC, and SOD levels of the heart tissue. Cardiotoxicity was confirmed both histologicaly and biochemically. WIT treatment ameliorated oxidative damage and protected against cardiotoxicity induced by DXR in rats. The protective effect of WIT may be mediated through its antioxidant, anti-inflammatory, and calcium-antagonistic properties.

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