ACADEMIC PRESS

Pharmacological Research 48 (2003) 461–465

Pharmacological research

www.elsevier.com/locate/yphrs

Influence of *p*-coumaric acid on doxorubicin-induced oxidative stress in rat's heart

M.H. Abdel-Wahab [a,*], M.A. El-Mahdy [a], M.F. Abd-Ellah [a], G.K. Helal [a], F. Khalifa [b], F.M.A. Hamada [a]

[a] *Pharmacology and Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt*
[b] *Biology Department, Faculty of Science, University of Kostantine, Kostantine, Algeria*

Accepted 16 June 2003

Abstract

The therapeutic value of doxorubicin (DOX) as anticancer antibiotic is limited by its cardiotoxicity. The implication of natural phenolic acids in the prevention of many pathologic diseases has been reported. Herein, the ability of *p*-coumaric (PC) acid, a member of phenolic acids, to protect rat's heart against DOX-induced oxidative stress was investigated. Three main groups of albino rats were used; DOX, PC, and PC plus DOX-receiving animals. Corresponding control animals were also used. DOX was administered i.p. in a single dose of 15 mg kg^{-1}. PC alone, in a dose of 100 mg kg^{-1}, was orally administered for five consecutive days. In PC/DOX group, rats received PC 5 days prior to DOX. DOX-induced high serum levels of lactic dehydrogenase (LDH) and creatine phosphokinase (CPK), were reduced significantly by PC administration, compared to DOX-receiving rats. Pretreatment with PC ameliorated the cardiac content of glutathione (GSH), and superoxide dismutase (SOD) & catalase (CAT) activities, compared to DOX-receiving rats. On the other hand, accumulation of cardiac content of MDA significantly decreased following PC pretreatment, compared to DOX-treated rats. The data presented here indicate that PC protects rats hearts against DOX-induced oxidative stress in the heart. It may be worthy to consider the usefulness of PC as adjuvant therapy in cancer management.

Keywords: Rat; Cardiotoxicity; Doxorubicin; Oxidative stress; Antioxidants; Phenolic acids; *p*-coumaric acid

1. Introduction

Doxorubicin (DOX) is a quinine-containing anticancer antibiotic that is widely used to treat different types of human neoplastic disease such as hematopoietic, lymphoblastic [1], and solid tumors [2]. However, its clinical value has been limited by dose-limited cardiotoxicity [3–5].

Several in vivo and in vitro studies have demonstrated that reactive oxygen metabolites including free radical species, superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) are important mediators of tissue injury [6]. The involvement of oxygen radical-induced injury of membrane lipids has been reported as the main causative factor for DOX-induced cardiotoxicity [7–10]. The cellular and biochemical changes involved in this process have been demonstrated. One-electron reduction of DOX leads to formation of the corresponding semiquinone free radical [11]. In the presence of oxygen, this free radical rapidly donates its electron to oxygen to generate superoxide anion ($O_2^{\bullet}$) [12]. The dismutation of superoxide yields hydrogen peroxide (H_2O_2) [13]. Under biological conditions, the anthracycline semiquinone or reduced metal ions such as iron reductively cleaves hydrogen peroxide to produce the hydroxyl radical which is the most reactive and destructive chemical species ever known [14]. This ultimately leads to lipid peroxidation, causing irreversible damage of membrane structure and function [15].

p-Coumaric acid (PC) is a phenolic acid widely distributed in plants and form a part of human diet [16]. The main dietary phenolic acids sources are fruits and beverages such as tea, coffee, wine, chocolate, and beer [17]. Attempts were made to explain the mechanisms of antioxidant effect of phenolics. These include the binding of metal ions, scavenging of reactive oxygen species (ROS); reactive nitrogen species (RNS); or their precursors, up-regulation of endogenous antioxidant enzymes, or the repair of oxidative damage to biomolecules [18]. Epidemiological studies have suggested association between the consumption of phenolic acids rich

* Corresponding author. Tel.: +20-202-4724450/4212.
E-mail address: elmahdym@hotmail.com (M.H. Abdel-Wahab).

1043-6618/$ – see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S1043-6618(03)00214-7

foods or beverages and the prevention of many diseases [19]. Recent interest in food phenolics has increased owing to their roles as antioxidants and scavengers of free radicals and their implication in the prevention of many pathologic diseases such as cardiovascular disease [18,29] and certain types of cancer [20]. In this study, we investigated the potential cardioprotective effect PC against DOX-induced oxidative stress in rat's heart.

2. Material and methods

Twenty four Swiss albino rats, weighing 180–200 g, were obtained from our local animal facility at Al-Azhar University. Rats were maintained in our facility under standard laboratory conditions (12:00-h-light/12:00-h-dark, 25 ± 2 °C) with free access to food and water.

Animals were divided equally into four groups, six animals each. First group, received solvent only, was served as control. Six animals received *p*-coumaric acid (Sigma Chemical Co. St. Louis, MO, USA) in a dose of 100 mg kg^{-1}, p.o. daily for five consecutive days. The third group received an i.p. injection of doxorubicin (Adriablastina Vials, Farmitalia Carlo Erba, Italy) alone at a dose of 4 mg kg^{-1} once. Another group of rats received PC acid five consecutive days prior exposure to DOX.

Twenty-four hours following last administration, the animals were sacrificed by cervical dislocation and the heart of each animal was rapidly dissected and washed with ice-cold 0.15 mM KCl to remove excess blood, then plotted between two filter papers and transferred into preweighed vials to determine the wet weight. Homogenization of each heart was carried out on ice using an Ultra-Turrax T25 homogenizer. Different aliquots of the heart homogenate were taken out and kept on ice for carrying out the following assays:

2.1. Assessment of cardiotoxicity

Determination of serum lactic dehydrogenase (LDH) and serum creatine phosphokinase (CPK) levels were assayed using commercially available reagents based on the method of Wacker et al. [21] and Buhl and Jackson [22]. Briefly, LDH and CPK specifically catalyze the oxidation of lactate to pyruvate and transphoshorylation of ADP to ATP, respectively, with subsequent formation of NADH. The rate at which NADH formed is proportional to enzyme activity. The increase in absorbance per minute, as a function of NADH production, is measured photometricaly at 340 nm.

2.2. Determination of antioxidant enzyme activities

The tissues (100 mg) were homogenized in 10 volume of ice-cold homogenization buffer (10 mM KH_2PO_4, pH 7.4; 20 mM EDTA;30 mM KCl). The homogenates were centrifuged at 40,000 × *g* for 30 min, and the supernatant was used to measure the activities of superoxide dismutase (SOD) and catalase (CAT). The activities of the enzymes were determined using concentrations of the tissue extracts in which the assays were linear and expressed as relative units per mg of protein.

SOD activity was determined by assessing the inhibition of pyrogallol autooxidation [23,24]. Pyrogallol (24 mmol l^{-1}) was prepared in 10 mM HCI and kept at 4 °C before use. Stock catalase solution (30 μmol l^{-1}) was prepared in phosphate buffer (0.1 M, pH 9). Aliquots of supernatants were added to Tris–HCl buffer (0.1 M, pH 7.8) containing 25 μl pyrogallol and 10 μl catalase. The final volume was adjusted to 3 ml using the same buffer solution. Changes in the absorbance at 420 nm were recorded every minute for 4 min.

CAT activity in the tissue homogenates was assayed according to the method of Aebi [25]. In this method, the decomposition of H_2O_2 (19 mmol l^{-1} H_2O_2 solution in potassium phosphate buffer; 10 mM, pH 7.4) due to CAT activity was assayed by the decrease in the absorbance of H_2O_2 at 240 nm.

2.3. Determination of reduced glutathione (GSH)

Because GSH accounts for the majority of soluble-reduced sulfhydryls in cells [26], reduced GSH in cardiac tissue was determined by measuring total soluble sulfhydryls content. GSH was determined by utilizing 5,5′-dithio-bis(2-nitrobenzoic acid) (Ellman reagent). Samples were prepared for non-protein fraction using equal volumes of 10% trichloroacetic acid solution. The protein precipitate was removed by centrifugation at 10,000 × *g* at 4 °C for 10 min. In the supernatants, sulfhydryl concentrations were determined photometrically following the method of Ellman [27].

2.4. Determination of lipid peroxidation

The measurement of heart lipid peroxide by a colorimetric reaction with thiobarbituric acid was done as described by Uchiyama and Mihara [28], and the determined lipid peroxide is referred to as malondialdehyde. Briefly, in a teflon-stoppered test tube, 2.5 ml of 20% trichloroacetic acid solution and 1 ml of 0.67% thiobarbituric acid solution were added to the homogenate. The color of thiobarbituric acid pigment was developed in a water bath at 100 °C for 30 min. After cooling with tap water to room temperature, 4 ml *n*-butanol was added and shaken vigorously. After centrifugation, the color of butanol layer was measured at 535 nm. The absorbance values obtained were compared against a standard curve of known concentrations of 1,1,3,3-tetraethoxypropane and normalized to the protein content of the specimen.

2.5. Determination of cardiac total proteins

Total protein was determined according to the method of Lowry et al [29].

2.6. Statistical analysis

Data are expressed as mean ± S.E.M. Data analysis was done using ANOVA followed by the Tukey–Kramer test for multiple comparison. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of p-coumaric acid on serum LDH and CPK activities

Single i.p. injection of DOX significantly elevated serum LDH and CPK levels reaching 423.5 ± 14.1 and 780 ± 15.7 U 1^{-1}, as compared with the control group. Pretreatment with PC significantly ameliorated LDH and CPK enzyme activities by 32.89 and 39.65%, respectively, compared with DOX receiving group. Solitary oral administration of PC did not significantly affect enzyme activities, when compared with the corresponding value of control group (Table 1).

3.2. Effect of p-coumaric acid on antioxidant enzyme activities

Administration of single i.p. dose of DOX significantly decreased the activity of cardiac SOD and CAT by 41.13 and 18.3%, respectively, compared with respective control. When PC was administered prior to DOX, the cardiac content of SOD and CAT activities were mitigated by 43.32 and 15.44%, respectively, compared to DOX alone. Five consecutive doses of PC did not significantly affect the activity of these enzymes, compared to control animals (Table 2).

3.3. Effect of p-coumaric acid on lipid peroxidation and GSH in cardiac tissue

Compared to normal control rats, solitary i.p. injection of DOX significantly decreased the cardiac content of GSH by 35%, however, the cardiac content of MDA was significantly elevated by 50%. Upon comparing the cardiac content of GSH and MDA in DOX/PC-receiving rats with those in DOX-receiving one, it was found that PC significantly enhanced the cardiac contents of GSH by 29.62%, however, the cardiac content of MDA was decreased and almost returned to its normal value. Oral administration of PC for five consecutive doses did not significantly change the cardiac content of MDA or GSH, compared to control animals (Table 3).

Table 1
Effect of *p*-coumaric acid (100 mg kg^{-1} per day, p.o. × 5), on doxorubicin (15 mg kg^{-1}, i.p.)-induced elevated serum levels of lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) activities

Parameters groups	LDH (U l^{-1})	CPK (U l^{-1})
Control	183. 9 ± 13.9	151.8 ± 22.3
PC treated group	171.1 ± 8.9	139.8 ± 11.70
DOX treated group	423.5 ± 14.1[a]	780.0 ± 11.57[a]
PC + DOX treated group	284.2 ± 11.65[b]	470.7 ± 26.7[b]

Data are expressed as mean ± S.E.M. of six rats per group.
[a] Denotes significant difference from control group at $P < 0.05$.
[b] Denotes significant difference from DOX treated group at $P < 0.05$.

Table 2
Effect of *p*-coumaric acid (100 mg kg^{-1} per day, p.o. × 5), on doxorubicin (15 mg kg^{-1}, i.p.)-induced elevated serum levels of super oxide dismutase (SOD) and catalase (CAT) activities

Parameters groups	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)
Control	12.35 ± 0.25	1101.7 ± 48.0
PC treated group	13.5 ± 0.36	1142.2 ± 65.1
DOX treated group	7.27 ± 0.28[a]	900 ± 59.2[a]
PC + DOX treated group	10.42 ± 0.31[b]	1039 ± 52.4[b]

Data are expressed as mean ± S.E.M. of six rats per group.
[a] Denotes significant difference from control group at $P < 0.05$.
[b] Denotes significant difference from DOX treated group at $P < 0.05$.

Table 3
Effect of *p*-coumaric acid (100 mg kg^{-1} per day, p.o. × 5), on cardiac content of reduced glutathione (GSH) and malonyldialdehyde (MDA) following doxorubicin (15 mg kg^{-1}, i.p.) administration

Parameters groups	GSH (μmol g^{-1} tissue)	MDA (nmol g^{-1} protein)
Control	4.2 ± 0.09	57.7 ± 1.22
PC treated group	4.6 ± 0.17	53.2 ± 1.03
DOX treated group	2.7 ± 0.08[a]	86.3 ± 1.76[a]
PC + DOX treated group	3.5 ± 0.15[b]	55.6 ± 1.36[b]

Data are expressed as mean ± S.E.M. of six rats per group.
[a] Denotes significant difference from control group at $P < 0.05$.
[b] Denotes significant difference from DOX treated group at $P < 0.05$.

4. Discussion

Doxorubicin is a broad spectrum antibiotic used as a chemotherapeutic drug for treatment of different forms of human neoplastic disease [30]. However, the clinical use of this anticancer drug is greatly limited by its dose-dependent cardiotoxicity [31]. So, the present work was designed to investigate the potential cardioprotective effect of PC, as a member of phenolic acids. PC is widely distributed in plants and forms a part of human diet [19]. It is well documented that, long-term treatment by DOX causes irreversible, severe and potentially life-threatening cardiac damage [32]. The mechanisms involved in such toxicity have been documented by many investigators [7–10]. The involvement of oxygen free radical, superoxide radical, and oxidative stress have been strongly accepted as crucial factors in the pathogenesis of DOX-induced cardiac damage.

The results of the present study indicate that administration of DOX in a dose of 15 mg kg^{-1}, i.p. elevated the serum levels of LDH and CPK. This is well agreed by vast majority of investigators. Al-Harby et al. [33], reported

increased levels of LDH and CPK after a single administration of DOX in a dose level of 15 mg kg^{-1} in rats. Similarly, Nagi and Mansour [34] and al Shabanah et al. [35] reported the cardiotoxicity of DOX in rats and mice. More invaluable observation in our study is that oral administration of PC, five doses of 100 mg kg^{-1}, prior to DOX significantly protects rats from DOX-induced elevated LDH and CPK levels. The epidemiological study that relates the low incidence of heart diseases and the generous intake of foods and beverage-containing phenolic acids strongly supports our finding. Additionally, prevention of a wide range of toxicities, including cardiotoxicity, of chemotherapeutic agents have been documented following administration of antioxidants such as melatonin and thymoquinone [34].

Assessment of lipid peroxidation, GSH content as well as SOD, CAT, and other antioxidant enzyme activities in cardiac tissue have been always used as markers for tissue injury and oxidative stress. Herein, cardiotoxicity and oxidative damage induced by DOX administration are also manifested by a significant increase in the activities of antioxidant enzymes, SOD, and CAT, the cardiac content of MDA, and a significant decease of GSH. It is already known that the heart is highly susceptible to oxidative stress due to its inherent decreased detoxifying natural antioxidants [36]. Also, the modulation of antioxidant enzyme activities following DOX administration has been discussed in many studies [7–10]. The association between elevated cardiac content of MDA and lowered cardiac content of GSH, found in this study, strongly proves the oxidative damage caused by DOX. This observation has been supported by the findings of Lazzarino et al. [37] and Gustafson et al. [36] who reported that, the cardiac content of MDA was increased and GSH content was reduced by administration of DOX to rodents. More interestingly, pretreatment of animals by five consecutive doses of PC markedly modulates the oxidative damage induced by DOX administration. This modulatory effect of PC would be, at least, in part due to it antioxidant scavenging potential. Many attempts were made to prevent the cardiotoxicity of DOX by other antioxidants, such as probucol [38], vitamin A [39], Q10 [40], alpha tocopherol [41], hydroxyanisol [42], and *N*-acetylcysteine [43]. The crucial protective role of antioxidants against a wide range of free radical generating agents has become well accepted. Recently, we have shown that antioxidant, tertiary butylhydroquinone, protected mice from dibromoacetonitrile-induced testicular oxidative damage [44]. It has also been reported that the antioxidant thymoquinone protected different organs against oxidative damage induced by DOX [35], cis-platin [45], and carbon tetrachloride [46].

Obviously, our study proves the beneficial effect of the natural phenolic acid PC in protecting animals against DOX-induced cardiac oxidative damage. This protecting potential of *p*-coumaric acid could be due to its free radicals scavenging capability. On the basis of our findings, it may be worthy to suggest the concomitant administration of *p*-coumaric acid with DOX cancer chemotherapy.

References

[1] Hitchcock-Bryan S, Gelber RD, Cassady JR, Sallan SE. The impact of induction anthracycline on long-term failure-free survival in childhood acute lymphoblastic leukemia. Med Pediatr Oncol 1986;14:211–5.
[2] Bonadonna G, Zambetti M, Valagussa P. Sequential or alternating doxorubcin and CMF regimens in breast cancer with more than three positive nodes. Ten-years results. JAMA 1996;273:542–7.
[3] Booser DJ, Hortobagyi GN. Anthracycline antibiotics in cancer therapy. Focus on drug resistance. Drugs 1994;47:223–58.
[4] Saltiel E, McGuire W. Doxorubicin (adriamycin) cardiomyopathy. West J Med 1983;139:332–41.
[5] Young RC, Ozols RF, Myers CE. The anthracycline antineoplastic drugs. N Engl J Med 1981;305:139–53.
[6] Fantone JC, Ward PA. Polymorphonuclear leukocyte-mediated cell and tissue injury: oxygen metabolites and their relations to human disease. Hum Pathol 1985;16:973–8.
[7] Myers CE, McGuire WP, Liss RH, Ifrim I, Grotzinger K, Young RC. Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. Science 1977;197:165–7.
[8] Mimnaugh EG, Trush MA, Bhatnagar M, Gram TE. Enhancement of reactive oxygen-dependent mitochondrial membrane lipid peroxidation by the anticancer drug adriamycin. Biochem Pharmacol 1985;34:847–56.
[9] Sarvazyan NA, Askari A, Huang WH. Effects of doxorubicin on cardiomyocytes with reduced level of superoxide dismutase. Life Sci 1995;57:1003–10.
[10] Singal PK, Siveski-Iliskovic N, Hill M, Thomas TP, Li T. Combination therapy with probucol prevents adriamycin-induced cardiomyopathy. J Mol Cell Cardiol 1995;27:1055–63.
[11] Doroshow JH. Effect of anthracycline antibiotics on oxygen radicals formation in rat heart. Cancer Res 1983;43:460–72.
[12] Bachur NR, Gordon SL, Gee MV, Kon H. NADPH-cytochrome P450 reductase activation of quinone anticancer agents to free radicals. Proc Natl Acad Sci USA 1997;76:945–57.
[13] Nakamura Y, Othtaki S, Makino R, Tanaka T, Ishimura Y. Superoxide anion in the initial product in the hydrogen peroxide formation catalyzed by NADPH oxidase in porcine thyroid plasma membrane. J Biol Chem 1989;264:4759–61.
[14] Kalyanaraman B, Morehouse KM, Mason RP. An electron paramagnetic resonance study of the interactions between the adriamycin semiquinone, hydrogen peroxide, iron-chelators and radical scavengers. Arch Biochem Biophys 1991;286:164–7.
[15] Odom AL, Hatwing CA, Stanley JS, Benson AM. Biochemical determinants of adriamycin toxicity in mouse liver, heart and intestine. Biochem Pharmacol 1992;43:831–6.
[16] Scalbert A, Williamson G. Dietary intake and bioavilability of polyphenols. J Nutr 2000;130(8 Suppl):2073S–85S.
[17] King A, Young G. Characteristics and occurrence of phenolic phytochemicals. J Am Diet Assoc 1999;99(2):213–8.
[18] Ursini F, Tubaro F, Rong J, Sevanian A. Optimization of nutrition: polyphenols and vascualr protection. Nutr Rev 1999;57(8):241–9.
[19] Morton LW, Caccetta RA, Ruddey IB, Croft KB. Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. Clin Exp Pharm Physiol 2000;27:152–9.
[20] Hudson EA, Dinh PA, Kokubun T, Simmonds MS, Gescher A. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol Biomarkers Prev 2000;9(11):1163–70.
[21] Wacker WEC, Ulmer DD, Wallee BL. Metallo enzymes and myocardial infarction: malic and lactic dehydrogenase activities and zinc concentrations in serum. N Engl J Med 1956;255:444–56.
[22] Buhl SN, Jackson KY. Optimal conditions and comparisons of lactate dehydrogenase catalysis of the lactate to pyruvate and pyruvate to

lactate reactions in human serum at 25, 30 and 37 degrees C. Clin Chem 1978;24:828–31.
[23] Minami M, Yoshikawa H. A simplified assay method of superoxide dismutase activity for clinical use. Clin Chem Acta 1979;92: 237.
[24] Marklund SL. Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice. Mutat Res 1985;148:129–34.
[25] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
[26] Kosower NS, Kosower EM. The glutathione status of cells. Int Rev Cytol 1978;54:109–60.
[27] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;74:214–26.
[28] Uchiyama M, Mihara M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86: 271–8.
[29] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265–75.
[30] Myers CE, Chabner BA. Anthracyclines. In: Chabner BA, Collins JM, editors. Cancer chamotherapy: principles and practice. Philadelphia: J.B. Lippincott Co., 1990. p. 356–381.
[31] Samuel L. Dox-induced cardiotoxicity. Postgrad Med J 1995;71: 318.
[32] Tone J, Ganguly PK, Singal PK. Myocardial adrenergic changes at two stages of heart failure due to adriamycin treatment in rats. Am J Physiol 1991;260:H909–16.
[33] Al-Harby MM, Al-Gharaply NM, Al-Shabanah OA, Al-Bekairi AM, Osman AM, Tawfik HN. Prevention of doxorubicin-induced myocardial and haematological toxicities in rats by iron chelator desferrioxamine. Cancer Chemother Pharmacol 1992;31:200–4.
[34] Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. Pharmacol Res 2000;41:283–9.
[35] al Shabanah OA, Badary OA, Nagi MN, al Gharably NM, al Rikabi AC, al Bekairi AM. Thymoquinone protects against doxorubicin-induced cardiotoxicity without compromising its antitumor activity. J Exp Clin Cancer Res 1998;17:193–8.
[36] Gustafson DL, Swanson JD, Pritsos CA. Modulation of glutathione and glutathione dependent antioxidant enzymes in mouse heart following doxorubicin therapy. Free Radic Res Commun 1993;19:111–20.
[37] Lazzarino G, Vida AR, Mulieri L, Ratitio G, Marvelli I. Prevention by fructos-1.6-bisphosphate of cardiacdamage induced in mice by subchronic doxorubicin treatment. Cancer Res 1987;47:6511–6.
[38] Siveski-Iliskovic N, Hill M, Chow DA, Singal PK. Probucol protects against adriamycin cardiomyopathy without interfering with its antitumor effect. Circulation 1995;91:10–5.
[39] Tesoriere L, Ciaccio M, Valenza M, Bongiorno A, Maresi E, Albiero R, Livrea MA. Effect of vitamin A administration on resistance of rat heart against doxorubicin-induced cardiotoxicity and lethality. J Pharmacol Exp Ther 1994;269:430–6.
[40] Choe JY, Combs AB, Folkers K. Prevention by coenzyme Q10 of the electrocardiographic changes induced by adriamycin in rats. Res Commun Chem Pathol Pharmacol 1979;23:199–202.
[41] Geetha A, Sankar R, Marar T, Devi CS. Alpha-tocopherol reduces doxorubicin-induced toxicity in rats—histological and biochemical evidences. Indian J Physiol Pharmacol 1990;34:94–100.
[42] Vora J, Khaw BA, Narula J, Boroujerdi M. Protective effect of butylated hydroxyanisole on adriamycin-induced cardiotoxicity. J Pharm Pharmacol 1996;48:940–4.
[43] Doroshow JH, Locker GY, Ifrim I, Myers CE. Prevention of doxorubicin cardiac toxicity in the mouse by *N*-acetylcysteine. J Clin Invest 1981;68:1053–64.
[44] Abdel-Wahab MH. Testicular toxicity of dibromoacetonitrile and possible protection by tertiary butylhydroquinone. Pharmacol Res 2003;47:509–15.
[45] Badary OA, Nagi MN, al Shabanah OA, al Sawaf HA, al Sohaibani MO, al Bekairi AM. Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. Can J Physiol Pharmacol 1997;75:1356–61.
[46] Alam K, Nagi MN, Badary OA, al Shabanah OA, al Rikabi AC, al Bekairi AM. The protective action of thymol against carbon tetrachloride hepatotoxicity in mice. Pharmacol Res 1999;40:159–63.