RESEARCH COMMUNICATION

Protective Effects of Silymarin against Doxorubicin-induced Toxicity

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Abstract

Objectives: The aim of the present study was to investigate the effect of silymarin on doxorubicin-induced toxicity to the rat kidney, heart, and liver. Materials and methods: A single dose of 10 mg/kg doxorubicin was injected intraperitoneally (ip) in the doxorubicin group. The silymarin group received silymarin (100 mg/kg) every other day. In the doxorubicin + silymarin group, silymarin was injected ip at 100 mg/kg dose for 5 days before doxorubicin administration (10 mg/kg, single ip injection) and then continued daily thereafter until euthanization. On the seventh day after doxorubicin injection, eight animals from each group were decapitated and liver and heart samples were obtained. The remaining eight animals of each group continued to receive silymarin every other day, till euthanized on the twenty first day. Serum was separated for determination of superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase (CAT), malondialdehyde (MDA), nitric oxide (NO), creatinine, urea, AST, ALT, lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) activities. Histopathological and electron microscopic examinations of heart, kidney and liver sections were also performed. Results: Doxorubicin caused a significant increase in serum NO levels compared to controls. Silymarin pretreatment group lowered these. Histopathological and electron microscopic examinations of kidney, heart, and liver sections showed doxorubicin to cause myocardial and renal injury which was levv evident in silymarin treated rats. Conclusion(s): Results of the present study indicate that silymarin significantly protected doxorubicin-induced toxicities to the rat kidney, heart, and liver, thus suggesting its administration as a supportive care agent during anti-cancer treatment featuring doxorubicin.

Keywords: Doxorubicin - silymarin - silibinin - *Silybum marianum* - milk thistle - cancer - toxicity

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Introduction

Doxorubicin is an antitumor anthracycline (ANT) antibiotic which is commonly used to treat a variety of cancers. The introduction of ANT antineoplastics to the chemotherapy of malignant neoplasms has been one of the major successes of cancer medicine. This is particularly evident in pediatric oncology, where the 5-year survival rate for childhood cancer has increased from 30% in the 1960s to 70–80% today (Gatta et al., 2002), while more than 50% of childhood cancer survivors have received ANTs (Krischer et al., 1997). The use of ANT in clinical chemotherapy is limited associated with progressive and clinically significant cardiotoxic effects (Lipshultz et al., 1991; 1995; 2005; Giantris et al., 1998; Grenier et al., 1998; Barry et al., 2007). Prevention of doxorubicin induced cardiotoxicity is particularly important in children because they are usually expected to survive for decades after treatment. Doxorubicin induced toxicities including renal (Bertani et al., 1982; Chen et al., 1998; Deman et al., 2001; Boonsanit et al., 2006) and hepatic (Ganey et al., 1988; Liu et al. 1992; Kalender et al., 2005; Yagmurca et al., 2007; Injac et al., 2009) injuries had been determined in experimental studies. The mechanisms of ANT-related toxicities are not yet fully understood. It may be because of lipid peroxidation and the generation of free radicals by ANT-iron complexes. One possible explanation for the cardiotoxicity of doxorubicin is the induction of reactive oxygen species (ROS) formation (Olson et al., 1990; Jeyaseelan et al., 1997; Barry et al., 2007). The heart is particularly vulnerable to injury from free radicals because it has a lower level of protective enzymes such as superoxide dismutase than other tissues (Doroshow et al.,. 1980; Keizer et al., 1990; Myers, 1998). The approaches to prevent of doxorubicin-related toxicities have centred on the use of antioxidants and chelators to minimize the generation of ROS.

Silymarin is an active extract from the seeds of the plant milk thistle (*Silybum marianum*), and contains approximately 65–80% silymarin flavonolignans (silymarin complex) with small amounts of flavonoids and approximately 20–35% fatty acids and other polyphenolic compounds. The major component of the silymarin complex is silybin that is synonymous with silibinin

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(Kroll et al., 2007). Silymarin is most well known for its antioxidant and chemoprotectant effects on the liver (Post-White et al., 2007). Beside liver cells, many other cells have proven to be sensitive to the protective action against toxic agents, including kidney cells, cardiac myositis, immune system cells, neurons, endothelial cells, fibroblasts, and keratinocytes (Comelli et al., 2007).

In addition, silymarin also showed anti-inflammatory, anti-proliferative, apoptosis-inducing, cell cycle–modulating, antiangiogenic and antimetastatic effects. Recent studies have suggested that, silymarin has also been shown to exert significant anti-neoplastic effects in a variety of in vitro and in vivo cancer models, including skin, breast, lung, colon, bladder, prostate and kidney carcinomas (Li et al. 2008; 2010; Ramasamy et al., 2008; Cheung et al., 2010).

The present studies investigate protective effects of silymarin during doxorubicin-induced toxicities to the rat kidney, heart, and liver.

Materials and Methods

Animals

The study was performed on female Wistar albino rats (n=72), 8–10 weeks of age and weighed ranging 200–250 g that were bred and kept at the animal center of Adnan Menderes University. They were housed in ventilated rooms at a temperature of 24 ± 2 ˚C with a 12 h light/dark cycle and 60 ± 5% humidity; maintained on standard pellet diet (Lipton rat feed, Ltd; Pune) and water ad libitum throughout the experimental period. The experimental procedure was approved by the Medical Ethical Committee of the Adnan Menderes University School of Medicine.

Chemicals

Doxorubicin (Doxo-Teva 50 mg) was obtained as gift samples from Teva. Silymarin (S0292-50 G) and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co.

Experimental procedure

Experimental procedure was arranged according to literature (Deman, Ceyssens, Pauwels, Zhang, Houte, Verbeelen, and Van den 2001;El-Shitany et al. 2008;Mansour et al. 2006;Yilmaz et al. 2006) and a pilot study. Pilot experiments were conducted to determine doses and duration of exposure, including a non-toxic dose of silymarin and toxic dose of doxorubicin. The dose of doxorubicin was selected to induce liver injury with minimal lethality.

The rats were divided into five groups including 16 animals, except for the control group: 1) Control group (n=8) ; 2) Dimethyl sulfoxide (DMSO) group; 3)Doxorubicin group; 4) Silymarin group; 5) Doxorubicin+silymarin group. A single dose of 10 mg/kg doxorubicin was injected intraperitoneally (ip) in doxorubicin group. Silymarin dissolved in 2 ml DMSO was injected ip every other day with a dose of 100 mg/kg throughout the study. In doxorubicin + silymarin group, silymarin was injected ip at 100 mg/kg dose for 5 days before doxorubicin administration (10 mg/kg, single ip injection) and silymarin was continued daily thereafter until euthanization throughout the study. In control group rats, ip saline (2 ml) injection was performed. DMSO group rats received DMSO only (ip) (2 ml).

Sample collection and biochemical assays

On the seventh day after saline (control), DMSO, or doxorubicin injection, eight animals from each group were decapitated (were anesthetized with ketamin) then liver and heart samples were obtained. Rat blood was taken via heart puncture after opening the thoracic region for biochemical analysis. The remaining eight animals of each group continued to receive silymarin every other day, till euthanizated on the twenty first day when their kidney and blood samples were harvested.

Laboratory Measurements

Rat blood samples were collected into biochemistry tubes, centrifuged at 4000 rpm for 10 minutes at +4 ˚C. The serum was removed and stored at -80 ˚C for later studies. For erithrocyte superoxide dismutase activity (SOD), glutathione peroxidase (GPx) and glutathione (GSH) levels, the blood was collected into EDTA containing tubes. The hemolysates were obtained by the related methods. Rat liver tissues were homogenized in 50 mM phosphate buffer, pH 7.0 at +4 ˚C (w/v = 1/10) for the malondialdehyde (MDA) and Nitric Oxide (NO) assays. The hemolysates and homogenates were also stored at -80 ˚C with serums until analysis.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea and creatinine levels were measured by spectrophotometric methods with an auto-analyzer (C8000 Architect, Abbott, Abbott Park, IL, U.S.A.).

Measurement of Malondialdehyde (MDA)

Malondialdehyde (MDA), which is a measure of lipid peroxidation, was spectrophotometrically measured by using the thiobarbituric acid assay (Ohkawa et al. 1979). MDA formed a colored complex in the presence of thiobarbituric acid, which was detectable by measurement of absorbance at 532 nm. Levels were calculated as μmol/L for serum.

Measurement of Nitric Oxide (NO)

NO (nitrite + nitrate) was assayed by a modification of cadmium-reduction method as mentioned by Navarro-Gonzàlves (Navarro-Gonzalvez et al. 1998). The nitrite produced was determined by diazotization of sulphanilamide and coupling to naphthlethylene diamine. For the measurement of NO, a 400 μL sample was denatured by adding 80 μL 30% $ZnSO_4$ solution, stirring and then centrifuging at 10000g for 20 minutes at +4 ˚C. First, the Cd granules were activated using CuSO4 solution in glycine-NaOH buffer. Then, 100 μL of deproteinized samples and standards were added. This reaction, using pretreatment of samples to reduce nitrate to nitrite, can be accomplished by catalytic reactions using enzyme or Cd. The samples were analyzed spectrophotometrically using a microplate reader and quantified automatically against

KNO3 standard curve. Results were expressed as μM/L for serum.

Measurement of Erithrocyte Glutathione (GSH)

Total glutathione content was measured according to the method of Tietze (Tietze 1969). In brief, 0.5 mL sample or standard solution was mixed with 0.25 mL of 1 mol/L sodium phosphate buffer (pH 6.8) and 0.5mL 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 4mg/L in the phosphate buffer) for 5 minutes. Then, the absorbance was measured at 412 nm using a Shimadzu UV-160 spectrophotometer. Hemoglobin in erythrocyte lysates was estimated using the Complete Blood Count (CBC) technology, with Advia 2120i Hematology System (Siemens). Results were expressed as U/gHb.

Measurement of Erithrocyte Glutathione peroxidase (GPx)

Glutathione peroxidase activity was determined by the method of Paglia and Valentine (Paglia et al. 1967). A 50 μL supernatant was transferred to a 1mL quartz cuvet, containing 950 μL of the reaction mixture (Tris buffer, 50 mmol/L, pH 7.6, containing per liter, 1 mmol of Na2EDTA, 2 mmol of reduced glutathione, 0.2 mmol NADPH, 4 mmol sodium azide, and 1000 IU of glutathione reductase. The mixture was incubated 5 minutes in 37 ˚C. Then, the reaction was initiated by adding 25 μL of H2O2, 8.8 mmol/L (% 30). The decrease in NADPH absorbance at 340nm was followed for 3 minutes. The nonenzymic reaction rate (blank) was determined by substituting water for the supernatant. The decrease in NADPH absorbance was recorded. Hemoglobin in hemolysates was evaluated as described above. Activity in samples was normalized for hemoglobin content and expressed in U/gHb.

Measurement of Erithrocyte Superoxide Dismutase Activity (SOD)

Erithrocyte SOD activity was assayed by the method of Sun Y et al. (Sun et al. 1988). The final volume of the reaction systems is 3.0 ml and contains, per liter, 0.1 mmol of xanthine, 0.1 mmol of EDTA, 50 mg of bovine serum albumin, 25 lmol of NBT, 9.9 nmol of xanthine oxidase, and 40 mmol of Na2CO3 (pH 10.2). Monitoring the increase in absorbance at 560 nm followed the production of blue formazan. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% as described by Sun Y et al. Hemoglobin was evaluated as described above. Data were expressed as U/gHb.

Histopathological examinations

The tissues were fixed in 10% formalin, processed routinely embedded in paraffin, sectioned at 5 μ, stained with Heamatoxylen-Eosin. Six coded slides from each group were examined by an observer blinded to the treatments. The changes of the kidney, heart and liver tissues on the light microscopy were graded as follows: normal (0); mild (1); moderate (2); severe (3).

Electron microscopic examination

Tissue samples from kidney, heart and liver (about 1 mm3 in size) for transmission electron microscope were fixed in % 2,5 gluteraldehyde. The specimens were washed in phosphate buffer and subsequently submerged in a solution containing equal amounts of 2% osmium tetra oxide and phosphate for 1 h. The samples were then passed through series of graded alcohol solutions and afterwards left in propylene oxide for 10 minutes. The samples were embedded in Araldite-CY 212 and dodecanyl succinate anhydride (DDSA) for one night. The next day they were placed in gelatin capsules that had been filled with a combination of Araldite-CY 212, DDSA and benzyl dimethylamine (BDMD), and these were incubated in an autoclave, first for 24 h at 40 °C and then for 48 h at 60 °C. The specimen lumps were cut into semithin sections with a Reichert LK ultramicrotome. The thick sections of 0.5mm were then stained with toluidine blue and examined under Olympus BH-2 (Tokyo, Japan) light microscope. Ultra-thin sections of 120–100A ° were obtained from selected blocks mounted on copper grids, stained with uranyl acetate and lead citrate and examined using a Carl Zeiss Libra120 transmission electron microscope (TEM) and images were digitally photographed.

Statistical analysis

Experimental values are expressed as the mean ± Standard error on the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey test were used to compare the study groups. P values of less than 0.05 were considered statistically significant.

Results

Total five rats died in doxorubicin (n=3) and doxorubicin+silymarin (n=2) groups during the study. No mortality was observed in remaining groups. Single dose doxorubicin treatment (10 mg/kg) caused a significant increase in serum NO levels compared to controls. When compared with doxorubicin group, silymarin pretreatment group had lower NO levels on the 7th and 21st day, which were only significant on the seventh day (Table 1). There were no significant differences in the serum levels of AST, ALT, LDH, urea, creatinine, MDA, GSH, GPx, SOD between different groups tested after silymarin treatment. Also a single ip injection of doxorubicin did not induce significant changes in serum AST, ALT, LDH,

Table 1. Serum Nitric Oxide Levels of Groups

Time after treatment	Groups	Serum NO levels (μM/L)
7 days	Control	0.868 ± 0.193
	DMSO	1.046 ± 0.324
	Doxorubicin	5.581 ± 1.743[a]
	Silymarin	1.905 ± 0.721
	Doxorubicin+silymarin	2.524 ± 0.562[b]
21 days	Control	0.868 ± 0.193
	DMSO	1.768 ± 0.355
	Doxorubicin	3.076 ±0.845[c]
	Silymarin	0.867 ± 0.177
	Doxorubicin+silymarin	2.054 ± 0.638

[a]p = 0.008, Doxorubicin vs. control, DMSO, and silymarin groups; [b]p = 0.008, Doxorubicin vs. doxorubicin+silymarin group; [c]p = 0.010, Doxorubicin vs. control and silymarin groups

Table 2. Nephrotoxicity Scores in Control, DMSO, Doxorubicin, Silymarin and Doxorubicin+silymarin groups

Group	Number of Animals	Toxicity score 0		+1		+2		+3	
Control	8	8	100	0	0	0	0	0	0
DMSO	8	6	75	2	25	0	0	0	0
Doxorubicin[a]	5	0	0	0	0	2	40	3	60
Silymarin	8	6	75	2	25	0	0	0	0
Doxorubicin+ silymarin[b]	6	0	0	4	60	2	40	0	0

Toxicity score: normal (0); mild (1); moderate (2); severe (3). DMSO, Dimethyl sulfoxide; [a]P < 0.001 Doxorubicin vs. Control, DMSO, silymarin, and doxorubicin groups; [b]P < 0.001 Doxorubicin+silymarin vs. control, DMSO, and silymarin groups

Table 3. Cardiotoxicity Scores in Control, DMSO, Doxorubicin, Silymarin and Doxorubicin+silymarin Groups

Group	Number of Animals	Toxicity score 0		+1		+2		+3	
Control	8	8	100	0	0	0	0	0	0
DMSO	8	4	50	3	37.5	1	12.5	0	0
Doxorubicin[a]	8	0	0	3	37.5	5	62.5	0	0
Silymarin	8	6	75	2	25	0	0	0	0
Doxorubicin+ silymarin[b]	8	2	25	4	50	2	25	0	0

Toxicity score: normal (0); mild (1); moderate (2); severe (3). DMSO, Dimethyl sulfoxide; [a]Doxorubicin vs. Control, DMSO, silymarin, and doxorubicin+silymarin groups; [b]Doxorubicin+silymarin vs. control group

urea, creatinine, MDA, GSH, GPx, SOD levels.

Histopathological examination

In histopathological examination, normal architecture was observed in control animals whereas renal lesions including marked tubular degeneration, tubular necrosis, tubular cast, and inflammation and congestion changes in the kidney of doxorubicin treated animals were observed (Table 2) (Figure 1). Cardiotoxicity induced by doxorubicin was further assessed using of hematoxylin and eosin stained sections. Hearts from control groups showed regular cell distribution and normal myocardium architecture. Histological examination of the rat hearts from doxorubicin only treated animals revealed cytoplasmic vacuole formation, interstitial edema (Table 3) (Figure 2). The severity of the histological changes was significantly lesser in sections from animals treated with silymarin (Figure 2).

Histological examination of the rat livers from doxorubicin only treated animals revealed minimal congestion. Bile duct proliferation, hepatocyte degeneration or pleomorphism, or necrosis were not identified in any groups.

Electron microscopic findings

The kidney tissues were normal in control group (see Figure 3. Both glomeruli and tubules were ultrastructurally had normal blood—primary filtrate barrier, podocytes with regular distribution of foot processes, mitochondria

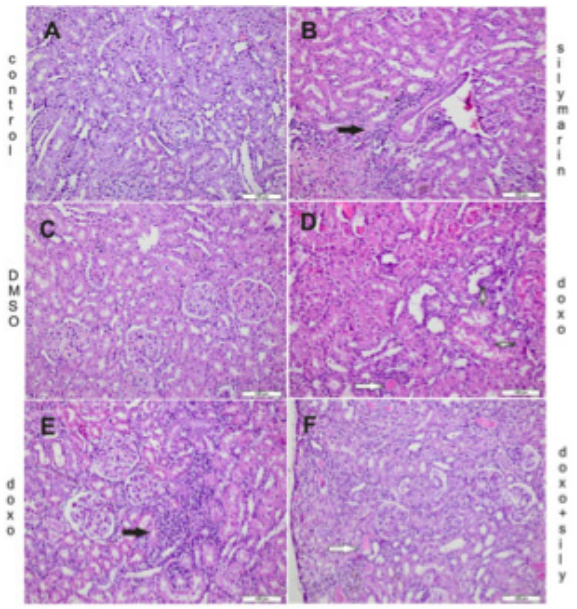

Figure 1. Light Micrographs of Rat Kidneys. A: Control group has normal kidney morphology, B: Treated with silymarin showing interstitial inflammation. D, E: Treated with doxo showing tubular cast, tubular degeneration and interstitial inflammation F: Treated with silymarin and doxo showing tubular cast (H&Ex200)

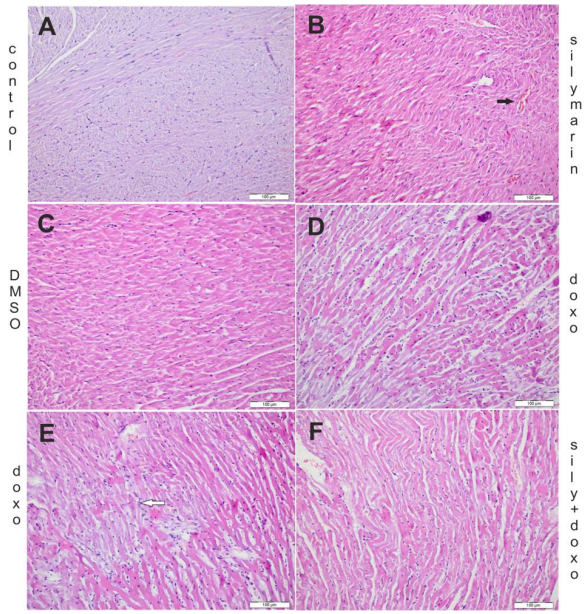

Figure 2. Light Micrographs of Rat Hearts. A: Control group has normal heart morphology. B: Treated with silymarin showing vascular congestion. D, E: Treated with doxo showing disorganisation and mild interstitial edema. F: Treated with silymarin and doxo showing minimal congestion (H&Ex200).

in cytoplasm visible and nuclei with normal chromatin. There were frequent and regular microvilli at the apical surface of the proximal tubules and had got normal intercellular distance between the proximal tubule epithelial cells. There were no morphological changes seen also in silymarin group and evaluated as normal. In DMSO group there were rare changes characterized at podocytes as fusion of foot processes. In the doxorubicin group the kidneys were showed significant and diffuse alterations of the glomeruli and tubules when compared to control group. These changes were characterized by fusion

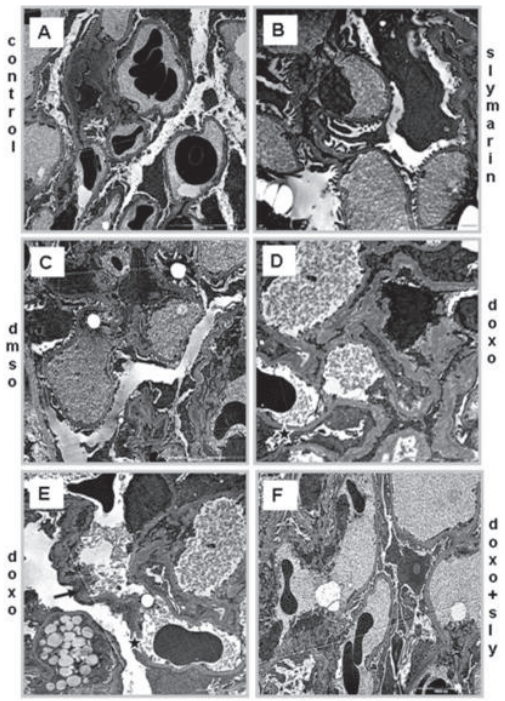

Figure 3. Kidney Ultrastructure. A: Control group has got normal kidney ultrastructure. B: Silymarin group shows normal kidney ultrastructure, C: In DMSO group ultrastructural findings are seen normal. D, E: Doxorubicin group shows fusion of foot processes of podocyte cells and in some areas podocytes with irregular distribution of foot processes are seen ( ), () indicating irregular basement membrane. F: Podocytes and basement membranes are preserved in doxorubicin+silymarin group.

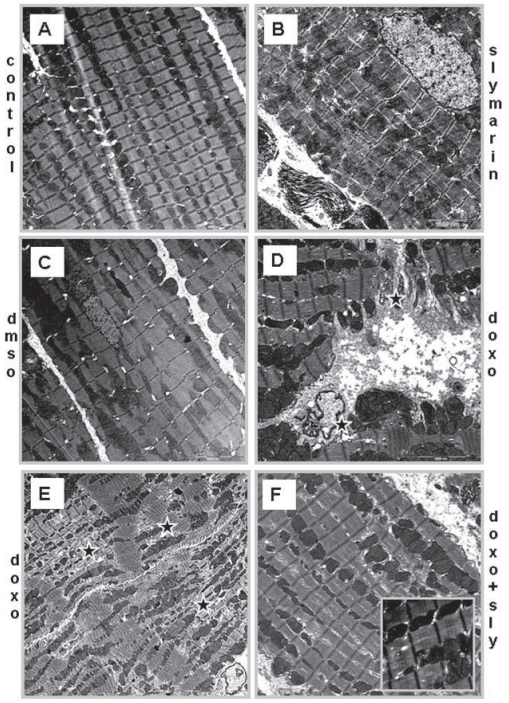

Figure 4. Heart Ultrastructure. A: Heart in control group reveals morphologically normal muscle ultrastructure. B: Silymarin group showed normal heart ultrastructure. C: In DMSO group ultrastructural findings were seen normal. D, E: In doxorubicin treated group, cross-sections reveals irregular myofibrillar disorganisation( ). F: Myocytes and mitochondrias are preserved in doxorubicin+silymarin group

of foot processes of podocyte cells. There were rare and irregular microvilli at the apical surface of the proximal tubules and widening of intercellular distance between the proximal tubule epithelial cells was seen. However, in the doxorubicin+silymarin group, there was a better preservation of foot processes than seen in doxorubicin group. Furthermore, in doxorubicin+silymarin group the distribution of glomeruler and tubuler lesion were clearly reduced when compared to the doxorubicin group.

The myocardial ultrastructure (see Figure 4) was evaluated as normal in control group. The myofibrils were parallel to the long axis of the cell with lateral alignment of the Z bands. There were no morphological changes seen also in silymarin group. In DMSO group there were rare dilated tubules of sarcoplasmic reticulum, nuclear chromatin and myofibrills were normal. In the doxorubicin group, myofibrillar disorganisations, disintegration and dilatation of sarcoplasmic reticulum were seen. In some areas irregular nuclear membrane and vesiculated rough endoplasmic reticulum were noticed. Cardiomyocytes generally had normal structure in the doxorubicin+silymarin group. Rare of the cardiomyocytes had myofibrillar loss and mitochondrial cristalysis. Intercalate discs, Z bands were normal. Rare dilated tubules of sarcoplasmic reticulum were present. Other structures, nuclear and myofibrillar formations were normal.

In control group hepatocytes (see Figure 4) had normal nuclei, the organelles in the cytoplasm; rough and smooth endoplasmic reticulum, golgi apparatus, ribosome, mitochondria and glycogen particles were intact and had

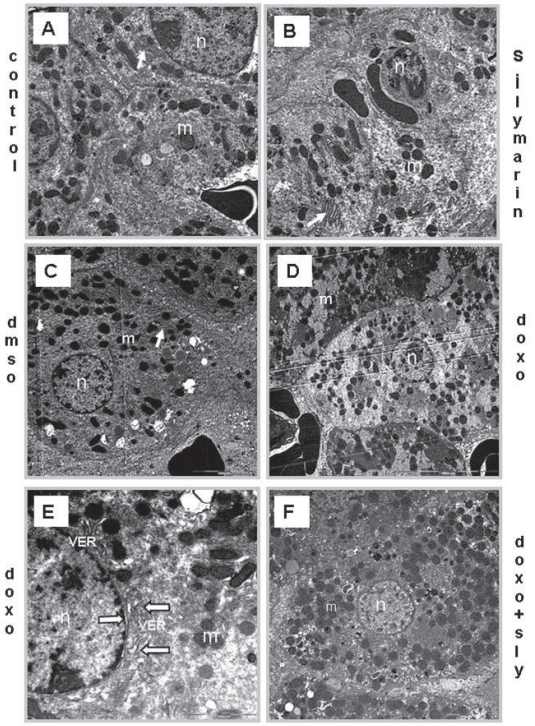

Figure 5. Liver Ultrastructure. A: In control group hepatocytes have normal nuclei (n), rough and smooth endoplasmic reticulum (white arrow), golgi apparatus, ribosome, mitochondria (m) and glycogen particles. B: There are no morphological changes in silymarin group and C: DMSO group D, E: In doxorubicin treated group, mitochondrial swelling, disintegration and dilatation of endoplasmic reticulum F: In doxorubicin+silymarin group, only weak mitochondrial swelling, disintegration and dilatation of endoplasmic reticulum

no ultrastructural changes. There were no morphological changes seen also in silymarin group. In DMSO group in some areas there were weak ultrastructural changes such as mitochondrial swelling and dilatation of endoplasmic reticulum. The main morphological changes were seen in the doxorubicin group. There were irregular mitochondria and endoplasmic reticulum cisternae. Mitochondrial swelling, disintegration and dilatation of endoplasmic reticulum were seen. In some areas irregular nuclear membrane and vesiculated rough endoplasmic reticulum were noticed. Silymarin treatment prevented these changes. The doxorubicin+silymarin group showed weak mitochondrial swelling, vesiculated rough endoplasmic reticulum, disintegration and dilatation of endoplasmic reticulum. Nucleus was normal in almost all areas.

Discussion

Our study implies the protective effects of silymarin on doxorubicin-induced toxicities to the rat kidney, heart, and liver. Doxorubicin administration was found to increase levels of NO significantly after 7 and 21 days. In the presence of silymarin, NO levels were significantly decreased. The contribution of NO on the cytotoxicity and organ toxicity of anticancer drugs have been previously reported (Wink et al., 1997; Sayed-Ahmed et al., 2001). Nitric oxide reacts spontaneously with the available superoxide radical to form the more potent and versatile oxidant peroxynitrite. This highly toxic species reacts with GSH, lipids, proteins and DNA. Moreover, NO production inactivates GSHPx activity via modification of a cysteine-like essential residue on GSHPx (Rhoden et al., 2001).

Liu et al. (2007) found that administration of doxorubicin (6.5 mg/kg, iv) caused an increase in MDA, apoptosis rate, urinary protein, BUN (blood urea nitrogen), NO, NOS (nitric oxide synthase), and serum creatinine, as well as decreases in GSH, SOD, and GST (glutathione-S-transferase) in rats when compared to the control group. Yagmurca et al. (2004) demonstrated that a 20 mg/kg (ip) single injection of doxorubicin caused increased SOD, MDA, NO, XO (xanthine oxidase), and MPO (myeloperoxidase) in kidney tissues in rats 10 days after administration. In our study, there were no significant differences in the serum levels of AST, ALT, LDH, urea, creatinine, MDA, GSH, GPx, SOD between different groups tested after silymarin treatment. Also a single ip injection of doxorubicin did not induce significant changes in serum AST, ALT, LDH, urea, creatinine, MDA, GSH, GPx, SOD levels. However, the histological findings demonstrate the severity of doxorubicin-induced cardiotoxicity and nephrotoxicity. The severity of the histological changes was significantly fewer in sections from animals treated with silymarin. Doxorubicin-only treated rats revealed simply minimal hepatic congestion on histological examination which was statistically insignificant. This is not concordant with the study of Yagmurca et al. (2004)that reported significant hepatotoxicity in doxorubicin-treated (20 mg/kg ip, single injection) group. They stated that the tissue of doxorubicin group showed some histopathological changes such as necrosis, hepatocyte degeneration, sinusoidal dilatation, hemorrhage and vascular congestion; however, we did not observe such changes with half intraperitoneal dose. The aforementioned histopathological changes were further strengthened by electron microscopic examination on kidney and heart tissues of rats. In spite of insignificant light microscopic findings, electron microscopic examination revealed prominent liver damage. Therefore, different results may be contributed to the difference in intraperitoneal doses.

Although the exact mechanism of doxorubicin-induced nephrotoxicity remains unknown, it is believed to be mediated through free radical formation, iron dependent oxidative damage of biological macromolecules and membrane lipid peroxidation (Pritsos et al. 2000). Several compounds with anti-oxidant activities are known to exhibit protection against doxorubicin-induced toxicities. In a preclinical study, silymarin prevented the renal damage usually caused by cisplatin in rats (Bokemeyer et al., 1996; Gaedeke et al., 1996; Karimi et al. 2005). Silymarin and its constituents protected rat heart microsomes and mitochondria against doxorubicin-induced lipid peroxidation, suggesting that the herb may possibly prevent doxorubicin mediated damage to the heart (Psotova et al., 2002). El-Shitany et al. (2008) found that, a single adriamycin dose (10 mg/kg) induced marked acute cardiotoxicity 72 h after adriamycin injection that was manifested by increased serum CPK and LDH activities and confirmed by severe histopathological changes in heart. Authors also reported that, silymarin pretreatment attenuated ADR-induced cardiotoxicity and nephrotoxicity. Protection of adriamycin-induced nephrotoxicity was manifested by deceased serum creatinine and urea levels and normal histopathological sections of adriamycin+ silymarin treated rats. They studied on the morphological changes by H&E dyed histopathological changes on light microscopy, but no scoring was reported.

Patel et al. (2010) found that, silymarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver. Present study demonstrated the protective effect of silymarin on doxorubicin induced hepatotoxicity by TEM. Recently, Ladas et al. (2010) reported the results of randomized, controlled, double-blind, multi-center pilot study of milk thistle (*Silybum marianum*) for the treatment of hepatotoxicity in children with ALL who were receiving maintenance-phase chemotherapy including methotrexate and 6 mercaptopurine. Authors found that, in children with ALL and liver toxicity, *Silybum marianum* was associated with a trend toward significant reduction in liver functionsand did not antagonize the effects of chemotherapy agents used.

In conclusion, results of the present study indicate that silymarin significantly protected doxorubicin-induced toxicities to the rat kidney, heart, and liver, thus suggesting its administration as a supportive care agent during anti-cancer treatment especially containing doxorubicin. Silymarin may have a great potential as a novel therapeutic agent to prevent toxic effects induced by doxorubicin. Further clinical studies are needed to determine the appropriate combination of silymarin with doxorubicin

to reduce doxorubicin-induced toxicities.

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The author(s) declare that they have no competing interests.

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