

## Effects of N-Acetylcysteine, Deferoxamine and Selenium on Doxorubicin-Induced Hepatotoxicity

Fatih Bulucu · Ramazan Ocal · Nuri Karadurmus · Mustafa Sahin · Levent Kenar · Ahmet Aydin · Cagatay Oktenli · Bayram Koc · Volkan Inal · Levent Yamanel · Halil Yaman

Received: 27 February 2009 / Accepted: 6 April 2009 /  
Published online: 25 April 2009  
© Humana Press Inc. 2009

**Abstract** The aims of our study were to evaluate the antioxidant defence mechanisms of liver tissue challenged by doxorubicin (DOX) and to compare the possible protective effects of *N*-acetylcysteine (NAC) ( $n=10$ ), deferoxamine (DOF) ( $n=10$ ), DOF+NAC ( $n=10$ ) and selenium ( $n=9$ ) on doxorubicin-induced hepatotoxicity. Fifty-six male rats (Mean weight =  $250\pm 50$  g) randomly divided into five groups. Animals in study groups were pretreated with a single dose of Dox, which was administered intravenously. Control group ( $n=7$ ) was treated with intravenous saline injection. Selenium was given intraperitoneally. Blood and urine samples were collected before sacrifice. Liver tissue samples were collected and tissue superoxide dismutase (SOD), glutathione peroxidase (GSH-px), CAT activity, MDA, Zn, iron and copper were determined. DOF decreased lipid peroxidation significantly. DOF and NAC decreased CAT activity significantly. Antioxidant regimes increase SOD activities significantly. DOF and NAC increase GSH-px activities and copper levels significantly. Beneficial effect of selenium seems to result from its stimulation of SOD but not to GSH-px. It has been found that DOF, NAC and selenium have protective effects on Dox-induced hepatocellular damage. DOF+NAC did not result additional benefit.

---

F. Bulucu · R. Ocal · N. Karadurmus · C. Oktenli · B. Koc  
Department of Internal Medicine, Gulhane Military Medical Academy, Ankara, Turkey

M. Sahin (✉)  
Department of Endocrinology, Gulhane Military Medical Academy, Ankara, Turkey  
e-mail: drsahinmustafa@yahoo.com

L. Kenar · H. Yaman  
Department of Biochemistry and Clinical Biochemistry, Gulhane Military Medical Academy, Ankara, Turkey

A. Aydin  
Department of Pharmaceutical Sciences, Gulhane Military Medical Academy, Ankara, Turkey

V. Inal  
Department of Internal Medicine, Military Hospital, Eskisehir, Turkey

L. Yamanel  
Department of the Intensive Care Medicine, Gulhane Military Medical Academy, Ankara, Turkey

**Keywords** Doxorubicin hepatotoxicity · Deferoxamine · N-acetylcysteine · Selenium · Rat

## Introduction

Doxorubicin (DOX), an anthracycline cancer drug, is widely used against a variety of human tumours [1]. However, clinical use of DOX in a sufficient dose is limited because of its dose-dependent form of myocardiotoxicity which is frequently lethal [2–4]. DOX also has been shown to produce hepatotoxicity [1, 5, 6]. It undergoes redox cycling leading to the production of oxygen radicals; DOX generates semiquinone radicals, which in turn react with molecular oxygen and provide other ROI at an early stage after administration. Semiquinone radicals react with molecular oxygen under aerobic conditions to form superoxide and hydrogen peroxide and, consequently, hydroxyl radical in the presence of transition metals (iron, copper (Cu)) [5]. It has been reported that DOX involve in antioxidant enzyme system, in liver tissue of guinea pigs [4, 7]. Some authors [4, 8] suggested that administration of a single dose of DOX decreases the content of cytochrome P-450 and glutathione (GSH) in rat liver while high levels of GSH were also found to protect isolated hepatocytes from DOX toxicity [4, 9].

N-acetyl L-cysteine (NAC), an aminothiol developed in the 1960s, is used as a mucolytic drug, because of its antimutagenic and anticarcinogenic qualities making it a potential chemopreventive agent. It acts by raising the intra-cellular concentration of cysteine, and hence of GSH, and/or acts by scavenging of oxidant species. NAC undergoes a rapid deacylation in the cells and provides a rate-limiting amino acid (cysteine) needed for intra-cellular synthesis of GSH, thereby replenishing the depleted levels of GSH in the target cells [10].

On the other hand, DOX free radicals come from a non-enzymatic mechanism that involves reactions with iron. For example,  $\text{Fe}^{+3}$  reacts with DOX in a redox reaction after which the iron atom accepts an electron and a  $\text{Fe}^{+2}$ -DOX free radical complex is produced. This iron-DOX complex can reduce oxygen to hydrogen peroxide and other active oxygen species [11–13]. In this context, the water-soluble iron chelators deferoxamine (DFO) is mainly used in non-iron overload conditions to produce antioxidant and antiproliferative effects [14].

Selenium is a vital trace element that in mammals exerts its most important function probably via selenium-dependent glutathione peroxidase. However, protective mechanisms of Se vary from organ to organ. Furthermore, high dosage Se-induced toxicity and even lethality are very well known [15, 16].

In our previous studies [17, 18] conducted to investigate the protective effect of some antioxidants in experimental nephrotic syndrome, livers of the rats were also harvested and then oxidative stress status in obtained liver tissues was studied later. The aim of the present article is to discuss both antioxidant defence mechanism of liver tissue challenged by DOX and other previous experimental studies performed in regard of investigation of DOX hepatotoxicity.

## Material and Methods

### Animals and Treatments

The study included 56 Sprague–Dawley male rats (mean weight  $250 \pm 50$  g). All rats were housed in pathogen-free conditions, with a 12 h light/dark cycle, using standard animal cages with free access to food and water. Five milligram per kilogram DOX (Adriblastina flacon, DEVA, Turkey) was injected intravenously via the tail vein under anaesthesia. Control rats ( $n=7$ ) were injected with an equal volume of isotonic saline. Five groups were built up from the

rats whose livers were harvested during our previous studies: one group given only DOX ( $n=17$ ) and four groups given DOX and an antioxidant treatment concurrently: (1) NAC ( $n=10$ ), (2) DFO ( $n=10$ ), (3) NAC plus DFO ( $n=10$ ) and (4) selenium ( $n=9$ ). Both NAC (Asist ampoule, Husnu Arsan, Turkey) and DFO (Desferal flacon, Novartis, Turkey) were administered in a dose of 20 mg/kg intravenously in single dose. Selenium yeast (obtained as powder from Kocak, Turkey, and a solution of concentration of 5 mg/ml in phosphate buffer was prepared) was given intraperitoneally at a dose of 15 mg/kg. All these agents were given immediately after DOX injections. The Animal Ethical Committee of Gülhane Military Medical Academy approved all animal procedures.

The spontaneously voided urine specimens from each animal were collected by using the metabolic cage for determination of protein and creatinine on the night before sacrifice. Blood samples were obtained by substernal cardiac puncture under anaesthesia. Blood samples were also drawn into tubes coated with Na-EDTA. One kidney and liver harvest from animals was realised immediately before they were sacrificed.

### Measurements

Blood chemistry tests such as, AST, ALT, ALP, bilirubines, total protein and albumin were analysed on autoanalyzer (Olympus AU 2700, Hamburg, Germany) and commercially available kits were used by using the methods according to the manufacturer's instructions. Urinary protein was measured by a colorimetric method with an Olympus AU 2700 autoanalyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Urinary creatinin was measured by modified Jaffe method with an Olympus AU 2700 autoanalyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Urinary protein to urinary creatinin ratio (PCR) was also calculated.

### Tissue Oxidative System Parameters and Trace Elements

Livers removed before sacrifice were stored at  $-80^{\circ}\text{C}$  until the assay performed for determinations of liver oxidative stress parameters in tissue homogenates. Tissues were homogenised with ice-cold KCl (1.15%) using a glass homogeniser. The homogenates was then centrifuged at  $4,400\times g$  for 10 min at  $4^{\circ}\text{C}$  to remove the cell debris and the supernatant obtained was used for the determination of antioxidant markers. Tissue SOD and GPX activities were measured on a UV-VIS Recording Spectrophotometer (UV-2100S, Shimadzu Co., Kyoto, Japan) as previously described [19]. Tissue SOD and GPX activities were expressed in units per gram tissue. Tissue CAT activity was measured in the supernatant at  $25^{\circ}\text{C}$  by the method of a previous report [20]. The activity was also expressed, as units per gram tissue.

For tissue specimens, supernatant MDA levels were determined in accordance with the method described in our previous study [20]. MDA levels of tissue samples were expressed as nmol/g tissue.

Tissue Zn, iron and Cu concentrations were determined by the atomic absorption spectrophotometer. Concentrations were expressed as microgram/gram.

### Statistical Analyses

Data were analysed with SPSS (SPSS Inc., Chicago, IL, USA) software. Descriptives were shown as the mean  $\pm$  SD notation. Normality of the parameters was tested with

Kolmogorov–Smirnov test. Parameter differences among the groups were investigated with one-way ANOVA and least significance difference (LSD) as the post hoc test.

## Results

The mean values of oxidative stress parameters and trace element levels determined in liver tissues of the rats are shown in Table 1. Table 2 shows the liver function tests studied in sera from animals.

Results indicate that DOX administration alone increased liver tissue MDA significantly ( $p < 0.001$ ). Both NAC ( $p = 0.001$ ) and selenium ( $p = 0.048$ ) ameliorated lipid peroxidation in liver tissue of DOX-injected rats. DFO also decreased lipid peroxidation significantly ( $p < 0.001$ ) (Fig. 1).

DFO alone ( $p = 0.023$ ) and combination with NAC ( $p = 0.025$ ) suppressed the tissue CAT activity significantly (Fig. 2).

Liver tissue SOD activities were suppressed in DOX-alone-given rats, compared to those of the controls ( $p < 0.001$ ). Fortunately, all studied antioxidant regimes in present study seemed to increase the liver tissue SOD activities significantly in DOX-treated animals ( $p < 0.001$  for NAC, DFO, NAC plus DFO and selenium groups) (Fig. 3).

DOX alone seemed to suppress liver tissue GSH-Px activities when compared to those of the controls ( $p = 0.004$ ). NAC ( $p = 0.001$ ) and DFO ( $p = 0.019$ ) also caused a significant increase in GSH-Px activities (Fig. 4).

Liver tissue Cu levels showed a statistically significant decrease in only-DOX-administered rats, compared to those of the controls ( $p < 0.001$ ). NAC ( $p < 0.001$ ), DFO ( $p = 0.006$ ) and NAC plus DFO ( $p < 0.001$ ) regimes provided significantly higher liver tissue Cu levels when compared to those of the only-DOX-given rats.

NAC plus DFO regime ( $p = 0.015$ ) increased liver tissue Zn levels in a statistically significant manner and selenium regime ( $p = 0.001$ ) caused significantly decrease in the same parameter values, when these two antioxidant regimes were compared to those of the only-DOX-administered rats. The increase in liver tissue Zn levels observed in NAC plus DFO regime rats led to significantly higher levels than those of the control rats ( $p = 0.001$ ).

Only-DOX-administered rats showed statistically significant lower liver tissue Fe levels than those of the controls ( $p = 0.005$ ). NAC plus DFO regime ( $p < 0.001$ ) and selenium ( $p = 0.016$ ) treatments increased the liver tissue Fe levels when compared with those of the rats administered DOX alone.

Serum ALT levels of the only-DOX-administered rats decreased significantly, compared to those of the controls ( $p = 0.020$ ). Both NAC ( $p = 0.010$ ) and DFO ( $p = 0.014$ ) provided higher ALT levels than those of the DOX alone group (Fig. 5). DOX administration led to significantly lower levels of serum AST, compared to those of the controls ( $p = 0.029$ ). Only DFO in studied antioxidant regimes in present study yielded an increase in AST levels when compared to those of the only-DOX group ( $p = 0.015$ ) (Fig. 6). Only-DOX administration reduced the serum ALP levels significantly ( $p = 0.003$ ) when compared to those of the controls. NAC ( $p < 0.001$ ), DFO ( $p < 0.001$ ) and selenium ( $p = 0.026$ ) provided higher ALP levels than those of the DOX-alone group rats (Fig. 7).

Only-DOX administration did not differ the rats from those of the controls in regard of serum total bilirubin and serum indirect bilirubin levels ( $p = 0.481$  and  $p = 0.796$ , respectively).

**Table 1** Tissue Antioxidant Enzyme Activities and Trace Element Levels in Groups

GROUPS	tMDA	tCAT	tSOD	tGSH	tCU	tZN	tFE
Controls	14.63±2.41	96.40±9.84	717.00±31.02	73.65±9.59	3.25±0.62	19.18±4.94	168.82±43.26
DOX alone	31.73±8.01 <sup>a</sup>	79.30±20.36	577.41±25.27 <sup>a</sup>	46.49±16.75 <sup>a</sup>	1.96±0.72 <sup>a</sup>	22.70±4.19	98.71±36.70 <sup>a</sup>
1 (NAC)	22.61±8.32 <sup>a</sup>	77.10±10.22	675.30±27.58 <sup>a</sup>	76.04±26.97 <sup>a</sup>	3.19±0.79 <sup>a</sup>	24.32±4.24 <sup>a</sup>	137.33±41.80
2 (DFO)	21.05±5.00 <sup>a</sup>	60.08±5.20 <sup>a</sup>	635.90±2.84 <sup>a</sup>	66.04±24.16 <sup>a</sup>	2.75±0.59 <sup>a</sup>	20.66±3.65	87.37±13.03
3 (NAC + DFO)	26.55±7.22	60.27±31.43 <sup>a</sup>	625.30±7.02 <sup>a</sup>	47.70±24.03	3.18±0.86 <sup>a</sup>	27.22±6.05 <sup>a</sup>	187.45±101.80 <sup>a</sup>
4. SEL	26.04±5.94 <sup>a</sup>	86.93±29.76	636.88±7.25 <sup>a</sup>	46.51±13.34	2.47±0.38	16.2±3.9 <sup>a</sup>	153.44±50.10 <sup>a</sup>

<sup>a</sup> Indicates the presence of statistical significance

**Table 2** Liver Function Tests in Sera Obtained from Study Animals

GROUPS	sALT	sAST	sALP	sTBIL	sINBIL	sTPRO	sALB	uPCR
Controls	81.28±26.41	246.28±162.19	337.00±60.45	0.13±0.02	0.11±0.02	5.85±0.22	2.92±0.11	15.1±10.8
DOX alone	60.94±22.09 <sup>a</sup>	154.41±86.84 <sup>a</sup>	196.76±142.72 <sup>a</sup>	0.12±0.03	0.11±0.05	5.27±0.49 <sup>a</sup>	2.10±0.35 <sup>a</sup>	92.4±57.4 <sup>a</sup>
1 (NAC)	82.5±13.77 <sup>a</sup>	213.00±112.09	369.00±61.56 <sup>a</sup>	0.14±0.03	0.10±0.04	6.00±0.35 <sup>a</sup>	2.77±0.18 <sup>a</sup>	12.3±8.6 <sup>a</sup>
2 (DFO)	80.10±10.45 <sup>a</sup>	245.80±63.03 <sup>a</sup>	365.70±57.33 <sup>a</sup>	0.10±0.05	0.08±0.04	5.84±0.56 <sup>a</sup>	2.53±0.28 <sup>a</sup>	34.4±27.5 <sup>a</sup>
3 (NAC + DFO)	71.60±17.39	189.00±77.88	220.30±110.97	0.09±0.04	0.08±0.04	5.25±0.52	2.02±0.25	67.4±18.5
4. SEL	66.00±18.12	175.22±20.58	291.11±64.97 <sup>a</sup>	0.11±0.04	0.09±0.04	5.77±0.43 <sup>a</sup>	2.54±0.46 <sup>a</sup>	62.3±57.3

<sup>a</sup> Indicates the presence of statistical significance

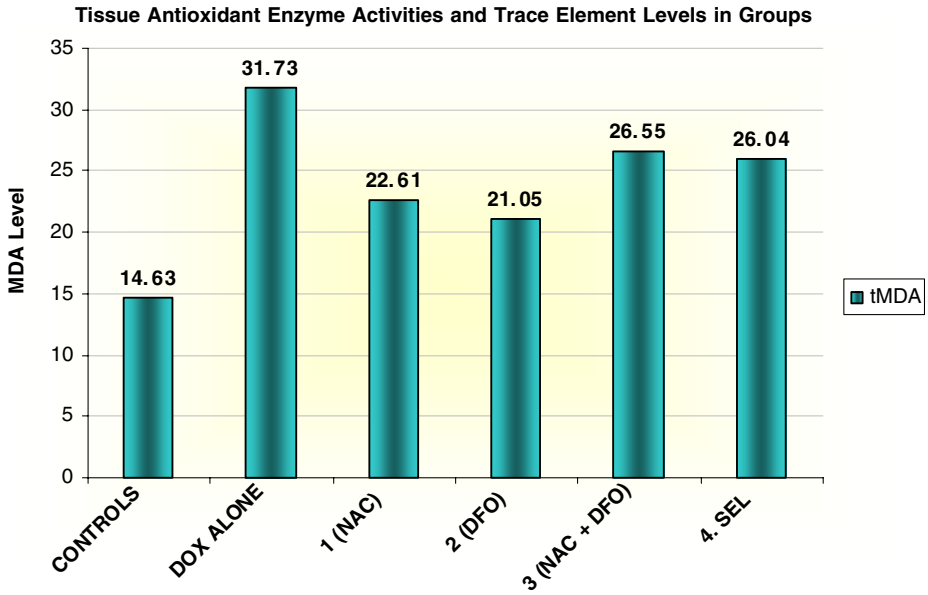


Fig. 1 Liver tissue MDA (malondialdehyde) levels in groups

Both serum total protein and albumin levels were significantly lower in only-DOX-administered rats when compared to those of the controls ( $p=0.008$  and  $p<0.001$ , respectively). NAC ( $p=0.001$  for total protein and  $p<0.001$  for albumin), DFO ( $p=0.004$  for total protein and  $p=0.001$  for albumin) and selenium ( $p=0.012$  for total protein and  $p<0.001$  for albumin)

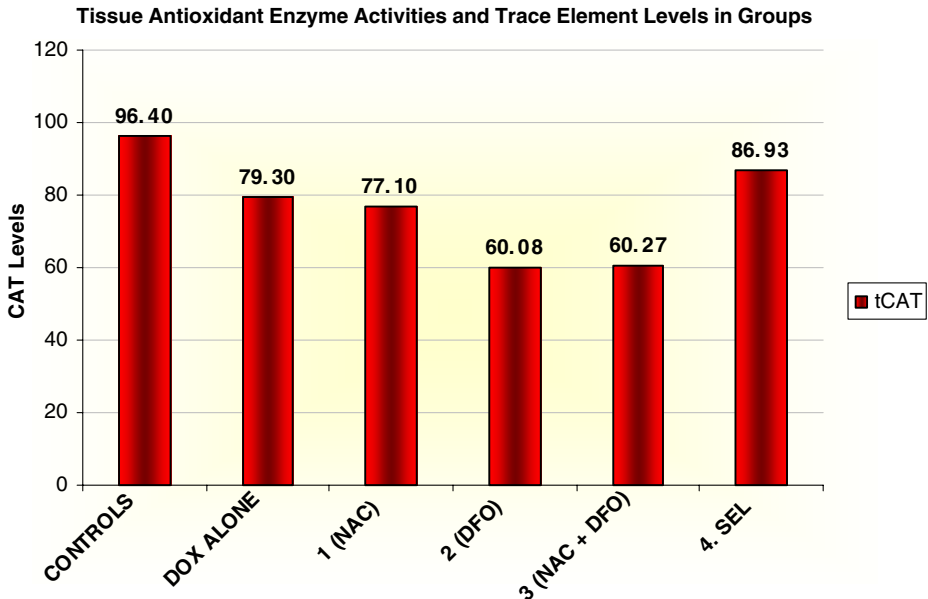


Fig. 2 Liver tissue catalase activity in groups

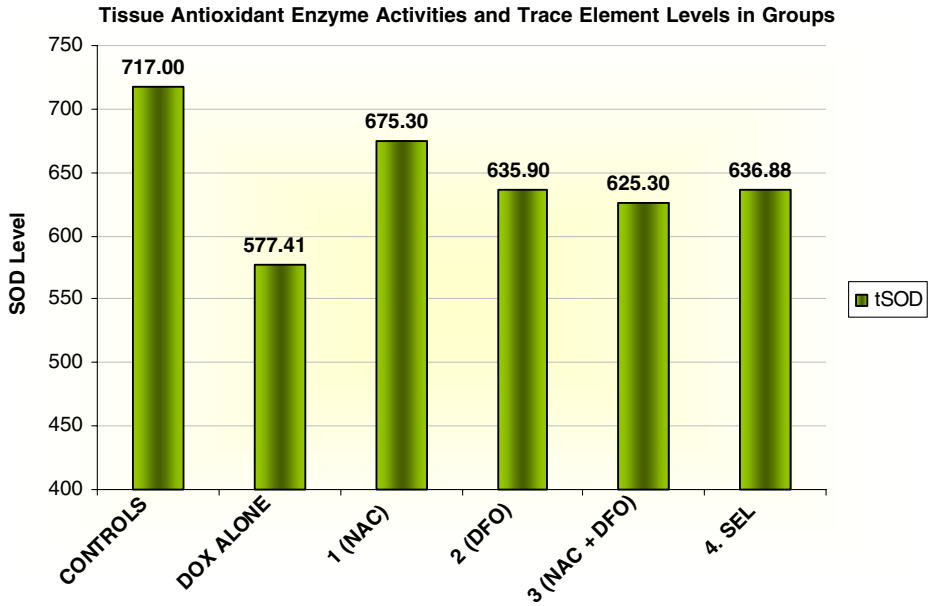


Fig. 3 Liver tissue SOD (superoxide dismutase) activity in groups

provided significantly higher levels of these parameters in rats administered these antioxidant regimes than those of the only-DOX-administered rats. NAC plus DFO regime did not establish a significant difference both in serum total protein ( $p=0.887$ ) and serum albumin ( $p=0.524$ ) levels when compared them to those of the only-DOX-treated group rats.

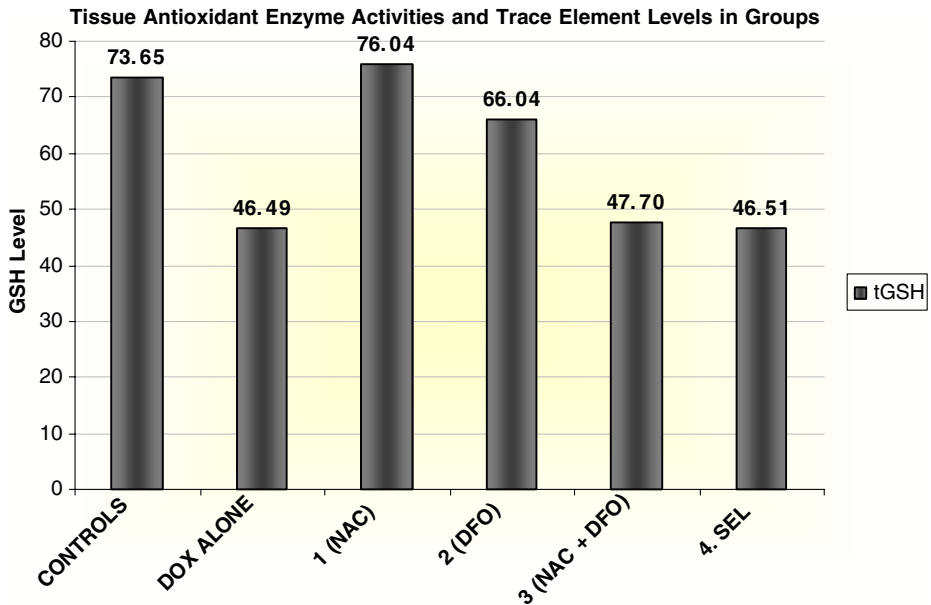


Fig. 4 Liver tissue GSH-Px (glutathione peroxidase) activity in groups



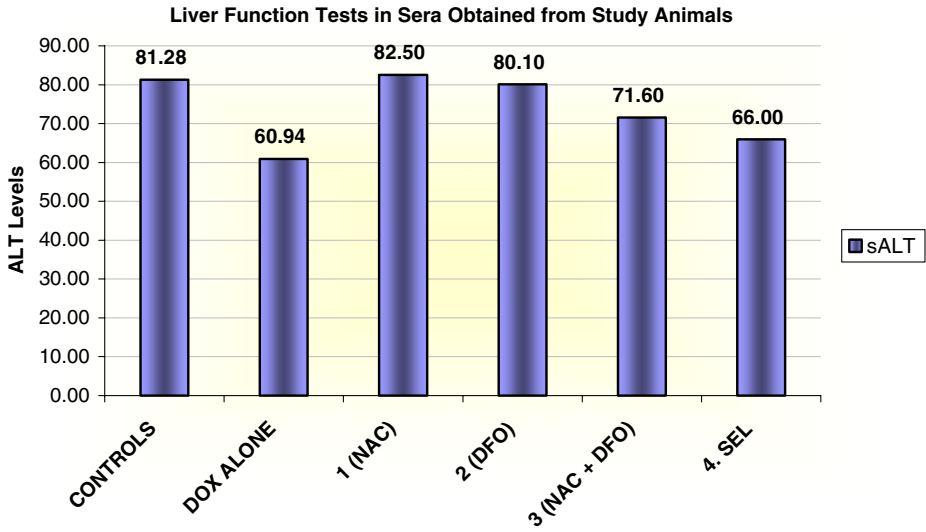


Fig. 5 Serum ALT levels in groups

DOX administration caused overt proteinuria when compared to controls ( $p < 0.001$ ). NAC ( $p < 0.001$ ) and DFO ( $p = 0.001$ ) were beneficial in reducing proteinuria in DOX-administered rats while NAC plus DFO ( $p = 0.142$ ) and selenium ( $p = 0.086$ ) were not effective with respect to this parameter.

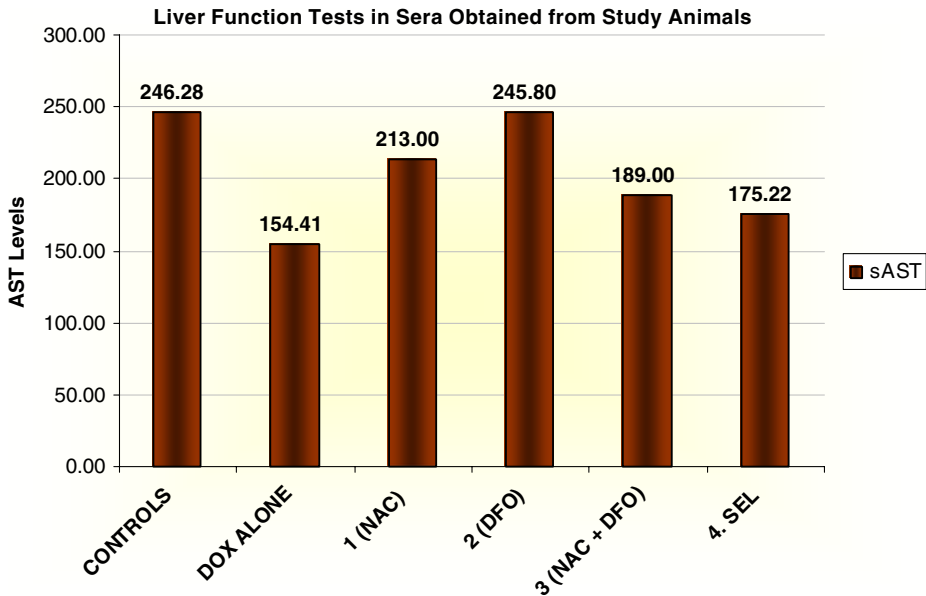
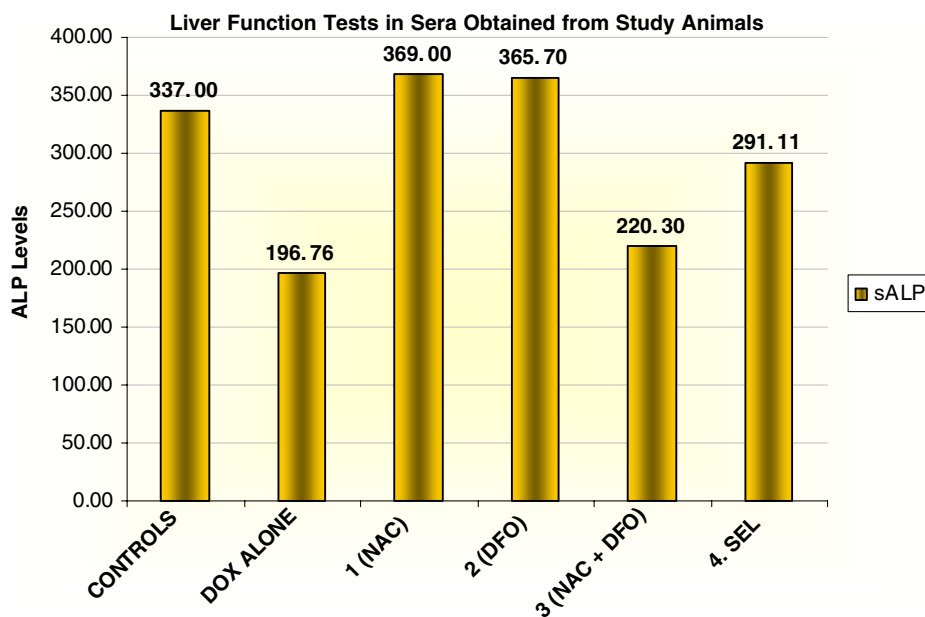


Fig. 6 Serum AST levels in groups



**Fig. 7** Serum ALP levels in groups

## Discussion

Although NAC can directly scavenge free radicals, the rate constants for their reaction with reactive oxygen species are several orders of magnitude lower than those of antioxidant enzymes such as SOD, CAT and GSH-Px. Thus, the direct free radical scavenging activity of NAC is not likely to be of great importance for its antioxidant activity *in vivo* [21]. In a histopathological study [22], NAC had no protective effects on DOX-induced hepatocellular damage. However, in present study, NAC seemed to have beneficial effects on DOX-induced lipid peroxidation in liver tissue of the rats by preserving the activity of SOD and GSH-Px enzyme activities.

*In vitro*, DFO is not only an iron chelator but also binds other metal ions, reacts with superoxide and hydroxyl radicals, affects eicosanoid synthesis, can act as a substrate for peroxidases and can generate a reactive nitroxide radical [23]. In our study, DFO was also observed to be effective in protecting the liver tissue of the rats against DOX-induced oxidative stress by preserving the activity of SOD and GSH-Px enzyme activities, as was in NAC treatment. In spite of deleterious effect of DFO on liver tissue CAT activity, its effective antioxidant property seen in this model was also remarkable. Because we could not be able to reach the studies conducted to investigate DFO antioxidant effects in DOX-induced hepatotoxicity, a comparison could not be realised.

Selenium is an essential trace element and its physiological role was established as a component of GSH-Px [24, 25]. This enzyme is also termed as a selenoprotein [26]. Selenium deficiency is usually associated with increased lipid peroxidation which affects cell functions [27–29]. In contrast to anticipated, the observed beneficial effect of selenium administration on preventing hepatic injury due to DOX in present study seemed to be resulted from its stimulation to SOD recovery but not to GSH-Px. Diphenyldiselenide, a

simple organoselenium compound, was reported to decrease methylmercury-induced hepatic oxidative stress. In that study, diphenyldiselenide did not modify hepatic GSH-Px, CAT and SOD activities and its beneficial effect was attributable to its preventive effect on the reduction in hepatic non-protein thiols (NPSH) levels [30]. Although it was reported that liver selenium-glutathione peroxidase was greatly diminished as a function of time in rats fed the selenium-deficient diets [31], both studies, ours and Freitas', established no beneficial effect of selenium supplementation on GSH-Px activity in rats exposed to oxidative stress. Because we could not also reach the studies investigating antioxidant effects of selenium in DOX-induced hepatotoxicity, a comprehensive comparison of our results could not be done, as was in DFO.

NAC plus DFO was found to be beneficial in preventing oxidative stress in previous studies investigated the role of this antioxidant regime in various organs of rats and mice, such as lung [32–34], brain [35–37] and liver [38, 39]. However, detrimental effects of *N*-acetylcysteine plus desferoxamine combination in an experimental nephrotic syndrome model were reported by us [17]. The harvested livers from the rats in the abovementioned and another study of us [18] were studied later for the present report. It was also noted that NAC plus DFO antioxidant combination regime was not successful to prevent lipid peroxidation in livers of rats administered DOX. This antioxidant combination seemed to cause a decrease in tissue catalase activity and an increase in tissue SOD activity while it was ineffective on tissue GSH-Px activity. The results of present study indicate that DFO interacts with ameliorating effects of NAC in rats exposed to DOX hepatotoxicity. When these two antioxidant agent were used together, iron released from iron stores due to action of DFO might interact with NAC [40]. Therefore, the anticipated prevention for oxidative stress with NAC plus DFO regime could not have been observed. Liver enzymes decreased after only DOX administration did also not recover under the NAC plus DFO antioxidant combination regime; however, these two antioxidants had beneficial effects on restoration of studied liver enzymes when used separately. This observation suggested an interaction between NAC and DFO.

Liver tissue trace element levels seemed to be independent from tissue oxidative stress status. Only DOX administration caused significantly decrease in tissue iron and copper levels but it did not change tissue zinc levels. No clear cut results could be obtained with respect to tissue trace element levels investigated under different antioxidant regimes in present study.

DOX administration caused significant decreases both in serum total protein and albumin levels and all types of antioxidant regimes yielded protective effects with respect to these parameters, except NAC plus DFO regime. Nevertheless, it should be kept in mind that harvested livers studied in present investigation were obtained from rats in nephrotic state. The effects of DOX administration on kidney, whether the animals developed nephrotic syndrome due to DOX or not, was not mentioned in the other experimental investigations [1, 4, 22, 41, 42] related to DOX-induced hepatotoxicity.

Finally, NAC, DFO and selenium have ameliorating effects on lipid peroxidation induced by DOX administration in liver tissue. NAC and DFO suggest to have an interaction between each other so that beneficial effects of NAC are hampered by concurrent use of DFO. Therefore, simultaneously use of NAC and DFO does not seem to be preventive against DOX-induced hepatic lipid peroxidation. Liver tissue SOD activity has an early recovery after DOX administration suggesting its independent behaviour from antioxidant regime effects. Furthermore, the probable effects of the presence of a nephrotic state should also be kept in mind in concluding the results of the studies investigating the DOX hepatotoxicity.

## References

1. Tomoki K, Fujita I, Itoh N, Muto N, Nakanishi T, Takahashi K, Azuma J, Tanaka K: Metallothionein acts as a cytoprotectant against doxorubicin toxicity. *JPET* 2000; 292:299–302.
2. Steinherz LJ, Steinherz PG, Tan CT, Heller G, Murphy ML: Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *JAMA* 1991; 266:1672–7.
3. Singal PK, Iliskovic N: Doxorubicin induced cardiomyopathy. *N Engl J Med* 1998; 339:900–905
4. Kalender Y, Yel M, Kalender S: Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin. *Toxicology* 2005; 209:39–45.
5. Ganey PE, Kauffmann FC, Thurman RG: Oxygen-dependent hepatotoxicity due to doxorubicin: role of reducing equivalent supply in perfused rat liver. *Mol Pharmacol* 1988; 34: 695–701.
6. Bagchi D, Bagchi M, Hassoun E, Kelly J, Stohs SJ: Adriamycin-induced hepatic and myocardial lipid-peroxidation and DNA damage, and enhanced excretion of urinary lipid metabolites in rats. *Toxicology* 1995; 95: 1–9.
7. Durak I, Öztürk HS, Kavutcu M, Birey M, Yel M, Guven T, Olcay E, Kacmaz M, Canbolat O: Protective role of antioxidant vitamins on adriamycin-induced free radical production and cardiotoxicity in guinea pigs. *Cancer Res Ther Cont* 1998; 5: 133–141.
8. Marchand DJ, Renton KW: Depression of cytochrome P-450-dependent drug biotransformation by adriamycin. *Toxicol Appl Pharmacol* 1981; 58: 83–88.
9. Babson JR, Abell NS, Reed DJ: Protective role of glutathione redox cycle against adriamycin-mediated toxicity in isolated hepatocytes. *Biochem Pharmacol* 1981; 30: 2299–2304.
10. Afaq F, Abidi P, Rahman Q: N-acetyl L-cysteine attenuates oxidant-mediated toxicity induced by chrysotile fibers. *Toxicol Letter* 2000; 117: 53–60.
11. De Beer EL, Bottone AE, Voest EE: Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. *Eur J Pharmacol* 2001; 415: 1–11.
12. Gianni L, Zweier JL, Levy A, Myers CE: Characterization of the cycle of iron-mediated electron transfer from adriamycin to molecular oxygen. *J Biol Chem* 1985; 260:6820–6826.
13. Sinha BK, Polliti PM: Antracyclines. *Cancer Chemother Biol Resp Modifier Ann* 1990; 11:45–57.
14. Voest EE, Vreugdenhil G, Marx JJM: Iron-chelating agents in non-iron overload conditions. *Ann Inter Med* 1994; 120: 490–499.
15. Stadtman TC: Selenium-dependent enzymes. *Annu Rev Biochem* 1980; 49: 93–110.
16. Stajn A, Zikic RV, Ognjanovic B, Saicic ZS, Pavlovic SZ, Kostic MM, Petrovic VM: Effect of cadmium and selenium on the antioxidant defence system in rat kidneys. *Comp Biochem Physiol* 1997; 117C:167–172.
17. Bulucu F, Oktenli C, Kenar L, Koc B, Ocal R, Karadurmus N, Inal V, Yamanel L, Sanisoğlu YS, Aydin A: Detrimental effects of N-acetylcysteine plus desferoxamine combination in an experimental nephrotic syndrome model. *Int J Toxicol*. 2007; 26:525–532.
18. Bulucu F, Oktenli C, Kenar L, Koc B, Ocal R, Inal V, Yamanel L, Yaman H, Sanisoğlu YS, Aydin A: Efficacy of deferoxamine, N-acetylcysteine and selenium treatments in rats with adriamycin-induced nephrotic syndrome. *J Nephrol* 2008; 21: 576–583.
19. Aydin A, Orhan H, Sayal A, Ozata M, Sahin G, Isimer A: Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. *Clin. Biochem.* 2001; 134: 65–70.
20. Aebi H. Catalase in vitro. *Methods. Enzymol* 1984; 105: 121–6.
21. Atkuri KR, Mantovani JJ, Herzenberg LA, Herzenberg LA. N-Acetylcysteine—a safe antidote for cysteine/glutathione deficiency. *Curr Opin Pharmacol.* 2007; 7:355–359.
22. Gokcimen A, Cim A, Tola HT, Bayram D, Kocak A, Ozguner F, Ayata A: Protective effect of N-acetylcysteine, caffeic acid and vitamin E on doxorubicin hepatotoxicity. *Hum Exp Toxicol* 2007; 26: 519–525.
23. Halliwell B: Protection against tissue damage in vivo by desferrioxamine: what is its mechanism of action? *Free Radic Biol Med* 1989; 7:645–651.
24. Schwarz K, Foltz CM: Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. *J Am Chem Soc* 1957; 79: 3292–3293.
25. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG: Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 1973; 179: 588–590.
26. Low SC, Berry MJ: Knowing when not to stop: selenocysteine incorporation in eukaryotes. *Trends in Biochem Sci* 1996; 21: 203–208.
27. Stadtman TC: Selenium biochemistry. *Ann Rev Biochem* 1990; 59: 111–127.
28. Valko M, Morris H, Cronin MT: Metals, toxicity and oxidative stress. *Curr Med Chem* 2005; 12: 1161–1208.
29. El-Sharaky AS, Newairy AA, Badreldeen MM, Eweda SM, Sheweita SA: Protective role of selenium against renal toxicity induced by cadmium in rats. *Toxicol* 2007; 235: 185–193.

30. de Freitas AS, Funck VR, Rotta MS, Bohrer D, Mörschbacher V, Puntel RL, Nogueira CW, Farina M, Aschner M, Rocha JB: Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. *Brain Res Bull* 2009; 79: 77–87.
31. Fraga CG, Arias RF, Llesuy SF, Koch OR; Boveris A: Effect of vitamin E- and selenium-deficiency on rat liver chemiluminescence. *Biochem. J.* 1987; 242: 383–386.
32. Teixeira KC, Soares FS, Rocha LG, Silveira PC, Silva LA, Valença SS, Dal Pizzol F, Streck EL, Pinho RA: Attenuation of bleomycin-induced lung injury and oxidative stress by N-acetylcysteine plus deferoxamine. *Pulm Pharmacol Ther* 2008; 21: 309–316.
33. Pinho RA, Silveira PC, Silva LA, Luiz Streck E, Dal-Pizzol F, Moreira JCF: N-acetylcysteine and deferoxamine reduce pulmonary oxidative stress and inflammation in rats after coal dust exposure. *Environ Res* 2005; 99:355–360.
34. Ritter C, da Cunha AA, Echer IC, Andrades M, Reinke A, Lucchiari N, Rocha J, Streck EL, Menna-Barreto S, Moreira JC, Dal-Pizzol F: Effects of N-acetylcysteine plus deferoxamine in lipopolysaccharide-induced acute lung injury in the rat. *Crit Care Med* 2006; 34:471–477.
35. Aguiar AS Jr, Tuon T, Soares FS, da Rocha LG, Silveira PC, Pinho RA: The effect of N-acetylcysteine and deferoxamine on exercise-induced oxidative damage in striatum and hippocampus of mice. *Neurochem Res.* 2008; 33:729–736. Epub 2007 Oct 17.
36. Di-Pietro PB, Dias ML, Scaini G, Burigo M, Constantino L, Machado RA, Dal-Pizzol F, Streck EL: Inhibition of brain creatine kinase activity after renal ischemia is attenuated by N-acetylcysteine and deferoxamine administration. *Neurosci Lett.* 2008; 434: 139–43.
37. Valvassori SS, Petronilho FC, Réus GZ, Steckert AV, Oliveira VB, Boeck CR, Kapczinski F, Dal-Pizzol F, Quevedo J: Effect of N-acetylcysteine and/or deferoxamine on oxidative stress and hyperactivity in an animal model of mania. *Prog Neuropsychopharmacol Biol Psychiatry* 2008; 32:1064–1068.
38. Schnellmann JG, Pumford NR, Kusewitt DF, Bucci TJ, Hinson JA: Deferoxamine delays the development of the hepatotoxicity of acetaminophen in mice. *Toxicol Lett.* 1999; 106:79–88.
39. Ritter C, Reinke A, Andrades M, Martins MR, Rocha J, Menna-Barreto S, Quevedo J, Moreira JC, Dal-Pizzol F: Protective effect of N-acetylcysteine and deferoxamine on carbon tetrachloride-induced acute hepatic failure in rats. *Crit Care Med.* 2004; 32(10):2079–83.
40. Sweetman SC: Martindale. *The Complete Drug Reference*, 33 rd edition, 2002, The Pharmaceutical Press, London, pp: 1082.
41. Yağmurca M, Bas O, Mollaoglu H, Sahin O, Nacar A, Karaman O, Songur A: Protective effects of erdosteine on doxorubicin-induced hepatotoxicity in rats. *Arch Med Res* 2007; 38:380–385.
42. Doroshow JH, Locker GY, Myers CE: Enzymatic defenses of mouse hearth against reactive oxygen metabolites. *J Clin Invest* 1980; 65: 128–135.