African Journal of Biotechnology Vol. 6 (20), pp. 2303-2309, 18 October 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Studies on the effect of doxorubicin on MDA, NO_2, NO_3, Se-GSH peroxidase and SOD levels in albino rat tissues

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Accepted 27 September, 2007

Doxorubicin, a highly effective anticancer drug, produces cardiotoxicity, which limits its therapeutic potential. The mechanism of this cardiotoxicity has remained elusive. The use of this drug, however, continues to be limited by its dose-related and time interval toxicity. Reactive oxygen species are hypothesized to be a major factor in the toxicity of doxorubicin. The aim of this work was to investigate the effect of doxorubicin on dose-related and time interval in rat. The study utilized adult albino rats (120 ± 5 g). They were divided into 8 groups of 7 animals each and were kept under standard laboratory conditions. They had free access to commercial pellet diet and water. The room temperature was maintained at 20 ± 5°C. The study measured rat tissue MDA, NO_2, NO_3, Se-GSH peroxidase and SOD under Dox stress, and the results indicate that MDA and NO generated in Dox treated samples cause neuro, myo, hepato and renal toxicity. Since SOD and Peroxidase are scavenging molecules, increase in their levels in Dox treated samples may be one of mechanism to overcome Dox caused oxidative stress in Dox treated albino rat.

Key words: Albino rat, Doxorubicin, MDA, NO_2, NO_3, SOD.

INTRODUCTION

Doxorubicin (Dox), one of the first anthracyclines in clinical use, has a broad antitumour spectrum, and has been used against a wide variety of hematopoietic malignancies and solid tumours (Ogura, 2001). Reactive oxygen species are hypothesized to be a major factor in the toxicity of doxorubicin (Keizer et al., 1990; Zhou et al., 2001), and measures controlling this oxidative damage are widely appreciated. Previous studies have demonstrated that antioxidant compounds have some protective effects in doxorubicin cardiotoxicity (Ciaccio et al., 1993; Nowak et al., 1995; Xu et al., 2001). However, there is little or no literature as how Dox affect overall metabolism of experimental animals. To bridge the gap, the *in vivo* effect of Dox on rat tissue MDA, NO2, NO3, Se-GSH peroxidase and SOD levels were attempted in the current study.

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Acronyms: Dox- Doxorubicin; **MDA**- Malandialdehyde; **NO**- Nitric oxide; **NO_2** - Nitric oxide; **NO_3**- -Nitrate; **ONOO**- -Peroxynitrate; **SOD**- superoxydismutase; **Se-GSH-Px**- Selenium dependent peroxidase; **Se-GSPx**- selenium glutathione peroxidase

MATERIALS AND METHODS

Doxorubicin hydrochloride (Adrim) is a product from Dabur India Limited (Pharmaceutical division) 19, Industrial area, Baddi, Dist. Solan (Himachal Pradesh), India. The study utilized adult albino rats (120 ± 5 g). They were divided into 8 groups of 7 animals each and were kept under standard laboratory conditions. They had free access to commercial pellet diet and water. The room temperature was maintained at 20 ± 5°C. Group I - IV rats acted as control ones and received normal saline. Group V received 10 mg/kg/wt of Dox for 5 weeks (weekly doses, i.v.), Group VI received 10 mg/kg/wt of Dox over 10 weeks (weekly doses, i.v.), Group VII animals received 30 mg/kg/wt of Dox over 5 weeks (weekly doses, i.v.) and Group VIII received 30 mg/kg/wt of Dox over 10 weeks (weekly doses, i.v.). After 5 or 10 weeks of Dox administration, the control and experimental group of rats were individually anaesthetised with pentobarbitone (10 mg/kg) and were sacrificed, tissue like brain, heart, liver and kidney were isolated. They were placed in liquid nitrogen immediately after isolation and were stored at -80°C till used. The heart, liver and kidney tissues after isolation were blotted on a filter paper before placing them in liquid nitrogen. Whenever necessary blood was collected by cardiac puncture before isolation of tissues and was allowed to clot centrifuged at 1000 x g for 10 min and the serum thus isolated was stored at -20°C till used. The lipid peroxides were determined by the TBA me-

Table 1. Effect of different doses of doxorubicin on rat tissue malandialdehyde dehydrogenase activity levels (values expressed as µmoles formazan formed/ mg protein/h).

Name of the tissue	Control		Dose of Doxorubicin and time period (10 mg/kg)		Control		Dose of Doxorubicin and time period (30 mg/kg)	
	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks
Brain	210.44	208.07	221.05*	240.76*	208.25	209.23	238*	267.09*
SD	± 3.66	± 2.04	± 1.05	± 0.74	± 2.19	± 1.26	± 6.27	± 4.10
PC			5.04	15.71			14.28	27.65
Heart	190.40	191.14	209.11*	220.40*	192.14	190.26	226.81*	240.25*
SD	± 1.74	± 1.22	± 0.67	± 2.15	± 7.98	± 0.26	± 1.08	± 2.14
PC			9.82	15.30			18.04	26.27
Liver	340.21	338.72	358.55*	372.75*	341.60	341.05	369.75*	387.32*
SD	± 4.16	± 0.96	± 1.26	± 3.05	± 2.14	± 0.98	± 4.20	± 5.49
PC			5.39	10.04			8.24	13.56
Kidney	220.05	221.52	240.46*	251.49*	222.74	220.96	249.10*	263.22*
SD	± 2.72	± 3.59	± 4.13	± 3.67	± 1.22	± 3.75	± 1.05	± 2.14
PC			9.27	13.52			11.98	19.12

Each value is the mean ± SD of seven samples. SD =Standard deviation; PC = percent change over the control.
* P<0.001

Table 2. Effect of different doses of doxorubicin on rat serum nitrite (NO_2^-) levels (values expressed as mg NO_2^-/ml of serum).

Name of the tissue	Control		Dose of Doxorubicin and time period (10 mg/kg)		Control		Dose of Doxorubicin and time period (30 mg/kg)	
	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks
Serum	56.92	55.08	70.20*	86.34*	56.22	57.25	90.44*	115.27*
SD	± 2.63	± 1.24	± 0.65	± 4.07	± 0.87	± 1.22	± 0.88	± 5.08
PC			23.33	56.75			60.86	101.34

Each value is the mean ± SD of seven samples. SD =Standard deviation; PC = percent change over the control.
*P < 0.001.

Table 3. Effect of different closes of doxorubicin on rat serum nitrate (NO_3^-) levels (values expressed as mg NO_3^-/ml of serum).

Name of the tissue	Control		Dose of Doxorubicin and time period (10 mg/kg)		Control		Dose of Doxorubicin and time period (30 mg/kg)	
	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks
Serum	43.66	42.14	50.36*	56.49*	43.05	41.66	60.43*	72.90*
SD	± 3.42	± 0.96	± 1.08	± 2.38	± 1.28	± 0.62	± 0.75	± 2.14
PC			15.34	34.05			- 40.37	74.98

Each value is the mean ± SD of seven samples. SD =Standard deviation; PC = percent change over the control.
*P < 0.001.

thod of Hiroshi and Yagi (1979). NO_2^- and NO_3^- determinants were done according to the procedure of Guarner et al. (1993). The peroxidase activity was measured by the method of Chenna Reddy et al. (1981). Superoxide dismutase activity was measured by method of Beachamp and Fridovich (1971). Statistical significance of the data was analysed through two way ANOVA (analysis of variance), Student New man Keuls test and regression analysis (Zar, 1984). P value <0.001 was considered significant.

RESULTS AND DISCUSSION

The data in Tables 1, 2, 3, 4 and 5 shows the levels of MDA, NO_2^-, NO_3^-, Se-GSH peroxidase and SOD activities under Dox at administered doses of 10 or 30 mg/kg over 5 or 10 weeks period enhanced rat brain, heart, liver and kidney based MDA, NO_2^-, NO_3^-, Se-GSH peroxidase and

Table 4. Effect of different doses of doxorubicin on rat tissue Se-GSH peroxidase activity levels values expressed as

Name of the tissue	Control		Dose of Doxorubicin and time period (10 mg/kg)		Control		Dose of Doxorubicin and time period (30 mg/kg)	
	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks
Brain	0.092	0.091	0.098*	0.111*	0.093	0.092	0.701*	0.712*
SD	± 0.004	± 0.006	± 0.025	± 0.29	± 0.003	± 0.12	± 0.053	± 0.087
PC			6.25	21.77			653.50	673.50
Heart	0.174	0.172	0.192	0.193	0.172	0.173	0.701*	0.712*
SD	± 0.042	± 0.036	± 0.042	± 0.367	± 0.036	± 0.16	± 0.136	± 0.42
PC			10.34	12.20			307.55	311.56
			NS	NS				
Liver	0.201	0.198	0.221	0.241	0.198	0.212	0.543*	0.553*
SD	± 0.069	± 0.043	± 0.064	± 0.362	± 0.039	± 0.075	± 0.072	± 0.036
PC			9.95	21.71			174.34	161.71
			NS	NS				
Kidney	0.235	0.232	0.264	0.280	0.225	0.234	0.912*	0.924*
SD	± 0.35	± 0.074	± 0.062	± 0.147	± 0.63	± 0.039	± 0.163	± 0.025
PC			12.34	20.68			305.35	294.37
			NS	NS				

Each value is the mean ± SD of seven samples. SD =Standard deviation; PC = percent change over the control; NS = Not Significant
*P < 0.001.

Table 5. Effect of different doses of doxorubicin on rat tissue superoxy dismutase activity levels (values expressed as µg/mg protein for)

Name of the tissue	Control		Dose of Doxorubicin and time period (10 mg/kg)		Control		Dose of Doxorubicin and time period (30 mg/kg)	
	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks	5 weeks	10 weeks
Brain	24.05	24.22	29.65*	33.11*	24.08	24.10	33.12*	42.08*
SD	± 0.061	± 0.022	± 0.067	± 0.052	± 1.05	± 0.27	± 0.085	± 0.12
PC			23.28	36.70			37.54	74.31
Heart	22.72	22.60	29.42*	31.06*	22.55	22.62	31.25*	40.82*
SD	± 0.031	± 0.044	± 0.37	± 0.37	± 0.037	± 0.094	± 0.063	± 0.049
PC			29.48	37.43			38.58	30.45
Liver	20.26	20.24	23.05*	27.56*	20.11	20.42	26.79*	34.24*
SD	± 0.84	± 0.65	± 0.064	± 0.24	± 1.24	± 0.93	± 1.24	± 0.73
PC			13.77	36.16			33.21	67.67
Kidney	13.60	14.62	17.14*	19.22*	15.49	15.52	25.32*	34.08*
SD	± 0.41	± 0.49	± 0.22	± 0.36	± 1.51	± 0.37	± 0.42	± 0.086
PC			26.02	31.46			63.46	119.58

Each value is the mean ± SD of seven samples. SD =Standard deviation; PC = percent change over the control.
*P < 0.001.

SOD levels and the changes were found to be statistically significant over the control (Tables 1 - 5 and Figures 1 - 5). More elevation in Dox treated rat tissues levels were found to be in the group of rat tissues receiving 30 mg/kg of Dox over 10 weeks period.

Highly reactive free radicals such as superoxide and lipid peroxide were involved in the development of disorders such as inflammation and tissue injury following cardiac arrest or transplant surgeons (Eckl et al., 1993; Rao et al., 1997). These have accorded attention in the assessment of drug-induced toxic cellular effects (Ohkawa et al., 1979). NO is a small, membrane permeable free radical synthesized from guanidine nitrogen arginine by the enzyme, NO synthase. It is known that NO is a potent biological mediator molecule produced by variety of cells and organs. Its role as destructor of foreign bodies and acting in the

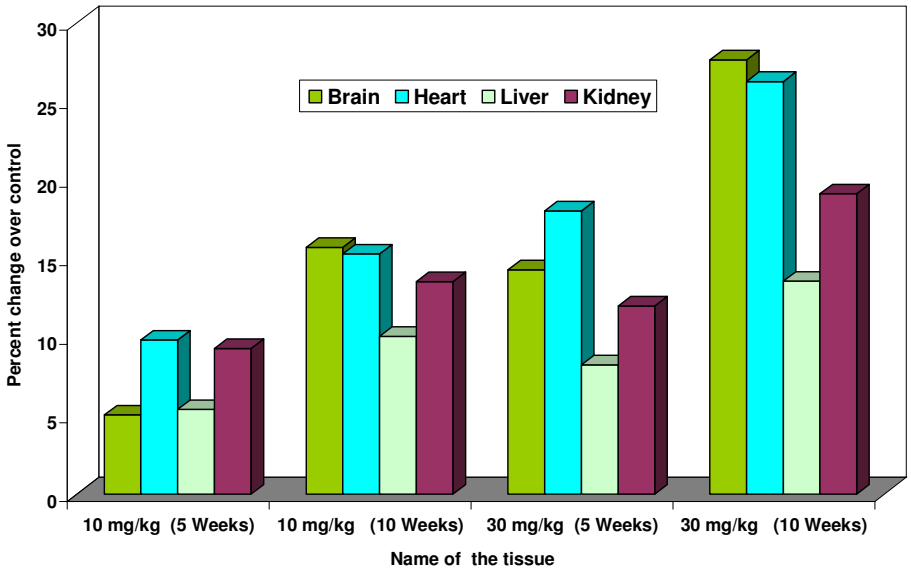

Figure 1. Doxorubicin induced percent changes of rat tissue malanoldialdehyde dehydrogenase activity level.

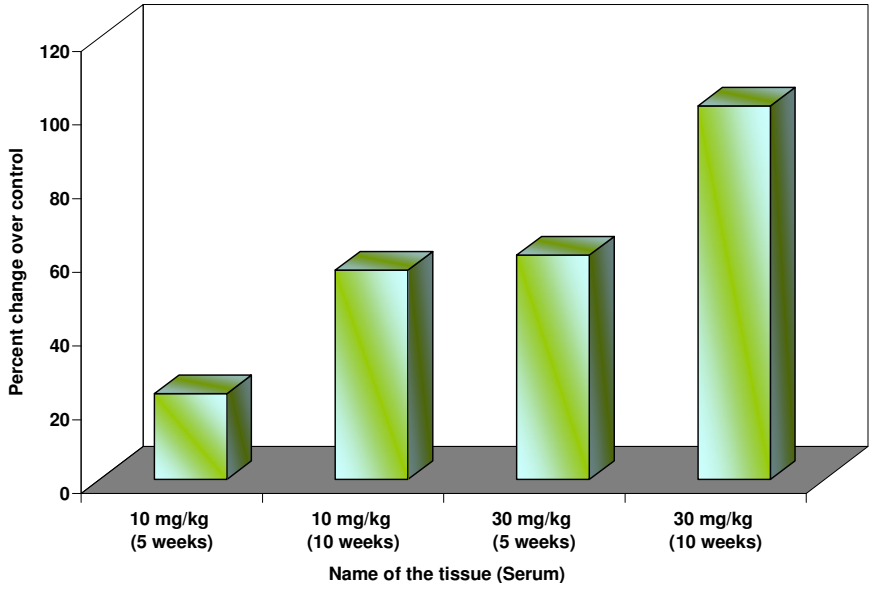

Figure 2. Doxorubicin induced percent changes of rat serum Nitrite (NO_2^-) Activity levels.

immune responses is well known. However, when produced in excessive concentrations, NO can, become toxic and may contribute to cell injury in many diseases (Laskin et al., 1994; Mathys and Bult, 1997). The dichotomy of NO is in part due to a broad array of redox species with distinctive properties and reactivity i.e., NO^+, NO and NO^- and it can be combined with superoxide anion radical (O_2^-) to yield peroxynitrite ($ONOO^-$) (Beckman et al., 1990). Under pathological stress conditions elevated levels of both NO and O_2 are produced by tissues. Because O_2 reacts with NO at a faster rate than with superoxide dismutase (SOD) as scavenger of O_2^-, $ONOO^-$ is formed and contribute for the pathology (Beckmann, 1996). $ONOO^-$ directly and rapidly oxidizes sulfhydral groups and initiates lipid peroxidation (Radi et al., 1991). This might be responsible for Dox induced lipid peroxide damage as reported by various authors (Myers et al., 1977; Doroshow et al., 1981; Karim et al., 2001). Alternatively, $ONOO^-$ can nitrate phenolic rings of tyrosine residue of proteins, leading to the formation of nitrotyrosine, which usually detected with anti-

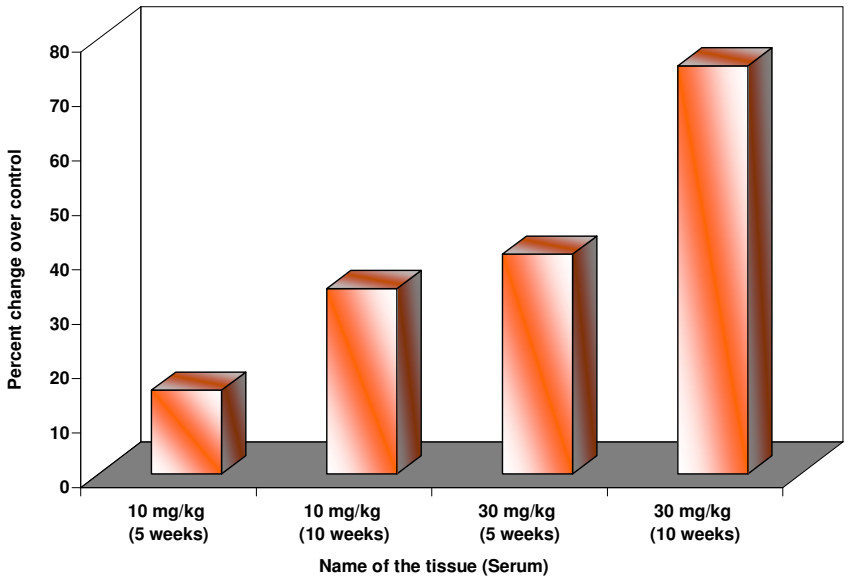

Figure 3. Doxorubicin induced percent changes of rat serum Nitrate (NO_3^-) activity level.

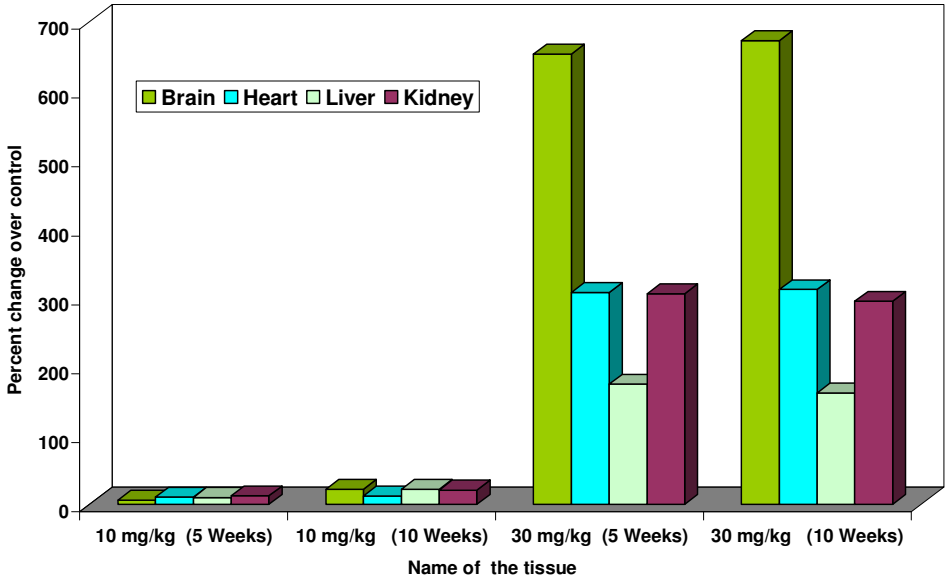

Figure 4. Doxorubicin induced percent changes of rat tissue peroxidase activity levels.

nitrotyrosine antibodies (Ischiropoulos et al., 1992). Thus the presence of nitrotyrosine can serve as $ONOO^-$ foot print. The free radical NO is well suited for its role in intracellular communications; its neutral changes allow free diffusion across biological membranes. In the presence of oxyhemoglobin, NO diffusing into the lumen of blood vessel will be very rapidly converted to nitrite, because of the high concentration of hemoglobin in the red blood cells. In the venous side of the circulations another reaction with deoxyhemoglobin also occurs (Wennmalam et al., 1992).

Superoxide act both as oxidants and reductants. Hence, these can modify a variety of biologically important modules (Fridovich, 1970). Superoxide radical (O_2^-) to form hydrogen peroxide. These distinct types of SOD have been described, which catalyze the same reaction with comparable efficiency (McCord et al., 1971). The cytosolic superoxide dismutase contains copper and zinc as prosthetic group where as the mitochondrial enzyme contains manganese as prosthetic group. Biological protection against O_2 is afforded by the SODs, which have a highly

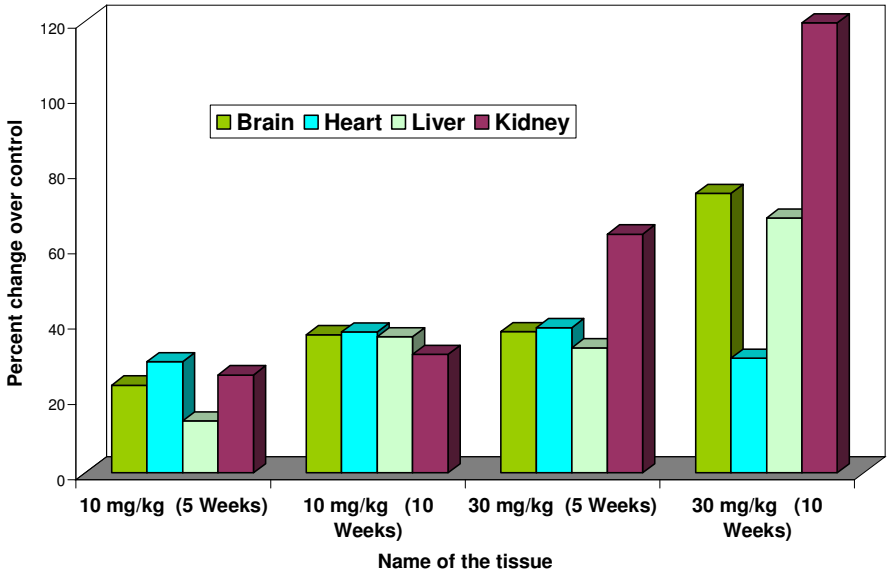

Figure 5. Doxorubicin induced percent changes of rat tissue superoxydismutase activity levels.

localized distribution. Selenium-dependent glutathione peroxidase contains a selenocystine residue at its active site that catalyzes the reduction of organic and inorganic hydroperoxides (Forstron et al., 1978). Glutathione peroxidase contains selenium as an integral component and capable of reducing lipid hydroperoxides and hydrogen peroxide (Rotruck et al., 1973). Se-GSH-Px is also involved in the reduction of fatty acid hydroperoxides generated during the production of prostaglandin, leukotrienes and related compounds via cycloxygenase and lipoxygenase pathways (Bruchhausen et al., 1988). Se-GSH-Px is known to be involved in the inhibition of cytoxygenase and lipoxygenase enzymes (Reddanna et al., 1989).

From the foregoing it is clear that from amongst the parameters studied, MDA, NO_2^- / NO_3^- are involved in generation of oxygen free radicals that damage cellular membranes and those of SOD and Se-GSH-Px act as scavengers of free radicals generated in the cells/tissues of animals under varied pathological conditions. There is adequate experimental evidence to support that Dox treatment produce oxygen free radicals and this is purely Dox dose and time-dependents, some important citations are reported. Dox at a dose of 20 mg/kg i.p./10 days enhanced kidney tissues MDA and nitric oxide levels and this was followed by an increase in Dox treated rat plasma GSH-Px activity and this leads to renal injury (Yagmurca et al., 2004). The report of Fadillioglu and Erdogan (2003) demonstrates that Dox at the tested dose of 20 mg/kg/12 days enhance the thiobarbutic acid reaction substances and this was accompanied by an increase in the plasma and erythrocyte SOD and GSH-Px activities. They further demonstrated that Dox treated rat erythrocyte NO levels were dramatically increased. The above were further supported by many authors (Ben-Shaul et al., 2001; Breitbart et al., 2001; El-Missiry et al., 2001; Abdel-Wahab et al., 2003; Dziegiel et al., 2003). However most of these studies explain Dox induced myocardial or renal toxicity with reference to the rats of generation of MDA or NO in Dox involved experimental models. In the present study more detailed experimental basis was provided to the extent that Dox at the tested doses of 10 or 30 mg over 5 or 10 weeks period enhance rat brain, heart, liver and kidney tissues including Dox treated rat serum NO_2^-/ NO_3^- levels. Since NO_2^- /NO_3^- are end products of NO pathway, this result support generation of more NO in Dox treated rat serum and both MDA and NO generated in Dox treated serum/tissues of rat may cause neuro-, myo-, hepato- and renal toxicity. Since SOD and GSH-Px are scavenging molecules, increase in their levels in Dox treated rat tissues and serum may be one of the mechanisms adopted by Dox treated albino rat to overcome the Dox caused oxidative stress and this may one of adaptive mechanisms exerted by the rat tissues under Dox stress.

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