BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2023. Vol. **20**(1), p. 293-305

Effect of the Anticancer Drug Doxorubicin (Adriamycin) on Antioxidant Studies and Ultrastructural Investigation in the Liver, Kidney, and Heart Tissues of Male Rats

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http://dx.doi.org/10.13005/bbra/3089

(Received: 04 January 2023; accepted: 03 March 2023)

Doxorubicin is a well-known antineoplastic agent that has proved to be successful in the treatment of various types of cancer. I used rats as the model to evaluate the effect of doxorubicin on antioxidant studies and ultrastructural investigations in the liver, kidney, and heart tissues. Male albino rats were given 1.0 mg/kg body weight of the anticancer drug doxorubicin intraperitoneally three times a week for 52 days. This was for a total of 18 doses. Control animals received 52 doses of 0.5 ml of saline over 52 days. The body weights of rats injected with doxorubicin experienced a significant decrease after the last dose compared to the control group of rats. In this study, the weights of the heart, kidneys, and liver were measured. Except for cardiac tissues, the protein content in the aforementioned tissues in treated rats was significantly different from the control. Glutathione (GSH) levels in the kidneys of experimental rats were not significantly lower (7.946 ± 0.781) compared to controls (8.06 ± 0.74) but there was a non-significant increase in GSH levels in the liver (17.095 ± 1.066) compared to controls (13.8 ± 1.3). In addition, the mean GSH levels in doxorubicin-treated hearts were significantly lower (7.9462 ± 0.781) compared to controls (8.06 ± 0.74). Lipid peroxidation (Lpx) and malondialdehyde content (MDA), a marker of lipid peroxidation, were found in much lower concentrations in the liver organ of the doxorubicin-treated group (0.0162 ± 0.00086) as compared to (0.20 ± 0.02) controls, and MDA content in the kidney was decreased (0.0239 ± 0.0003) compared to control rats (0.31 ± 0.03), as well as heat production (0.0398 ± 0.00097) compared to (47.451 ± 1.708) controls. Glutathione reductase (GR) levels were significantly elevated in the same tissue treatment group. Glutathione-S-Transferase (G-S-T) activity was assessed and significantly increased in all tissues in the doxorubicin model. Glutathione peroxidase (GPx) activity showed a significant decrease in all the above tissues after doxorubicin injection. The catalase (CAT) activity of doxorubicin was greatly increased in one treated rat. In the doxorubicin-treated group, levels of cytochrome p450 (CYTp450) were significantly decreased in liver and kidney tissue and significantly elevated in heart tissue. After doxorubicin treatment, cytochrome b5 (CYTb5) levels in liver tissues increased significantly (837.177± 61.197) compared to controls (615 ± 37.0), and the contents of cytochrome b5 in rats' kidneys increased significantly (447.685 ± 35.215) compared to controls (2605.5± 259.2). and cytochrome b5 in heart tissues was lower (165.352± 8.7) when compared to controls (88± 0.4). The results showed that there were few obvious changes in histological, ultrastructural, and biochemical changes in liver tissue in the doxorubicin model. Long-term doxorubicin treatment in kidney tissue results in no significant changes at the light microscopic level, but the electron microscopic level reveals no change from a histological point of view.

Keywords: Antioxidant; Antioxidant Enzymes; Doxorubicin; light microscopy; Kidney; Liver; Ultra structure.

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Doxorubicin (adriamycin), an antibiotic from the tetracyclic anthracycline class, was found in the cultures of *Streptomyces peucetiues var. caesius* [1,2,3]. Since the late 1960s, this has been one of the most efficient and popular chemotherapeutic medications[4,5]. It is a cytotoxic compound that interacts with mammalian cells to inhibit cell growth and viability at low concentrations[6,7]. It has a tetracyclic structure, which is linked to carbohydrates; removal of carbohydrates from it usually leads to loss of anti-tumor activity. In the treatment of cancers such as solid tumors, multiple myelomas, breast cancer, stomach cancer, ovarian cancer, testicular cancer, and bone cancer, doxorubicin is crucial[8,9,10]. Long-term use of this anticancer drug can lead to the accumulation of its metabolite, doxorubicinol, which can cause cardiomyopathy due to its cytotoxicity. This cardiotoxicity is dependent on the amount of doxorubicin and doxorubicinol accumulated in the body[11]. Doxorubicinol has stronger cardiotoxic effects than doxorubicin[12]. The mechanism of action of the drug is not entirely clear and may involve multiple actions[13]. The anti-tumor activity of doxorubicin can intercalate between DNA bases, interfering with DNA replication and the transcription of RNA. During metabolism, doxorubicin (quinone) is reduced by a two-electron reaction to semiquinone and then to hydroquinone. Semiquinones react with molecular oxygen under aerobic conditions to form superoxide and hydrogen peroxide, which in turn form hydroxyl radicals in the presence of transition metals [iron, copper][14, 15, 16]. Doxorubicin is classified as a Class III, so-called topoisomerase inhibitor. Double-stranded DNA can be either two loosely coiled molecules (negatively supercoiling) or two tightly coiled molecules (positively supercoiling) relative to its lowest energy state.[17]. The enzyme topoisomerase plays a key role in temporarily cutting one strand. As a result, the other strand can pass the breakpoint, resulting in clipping the ends together, which is the backbone of replication. Doxorubicin inhibits the ability of the topoisomerase II enzyme to rejoin the severed ends, causing DNA strand breaks throughout dividing cells, a process that ultimately leads to cell death.

In our study, we examined enzymatic and non-enzymatic changes in the liver, kidney, and heart of the doxorubicin model compared to controls. Organelle changes under the influence of the anticancer drug doxorubicin were also examined using electron and light microscopy. The current investigation is fundamentally focused on understanding the toxic effects of the commonly used anticancer drug doxorubicin on antioxidant structures and detoxification systems in experimental rat models. This is to monitor general health and aims to demonstrate the efficacy of this drug in albino rats. Become a rat while receiving treatment. Several new approaches are being explored to improve activity and reduce the unwanted side effects of chemotherapy.

MATERIALS AND METHODS

Animals

Verifiable fertile adult male albino rats of the Wistar strain weighing approximately 240-250 grams were obtained from the Haffkine Research Institute, Mumbai, India. Five animals in each control and treatment group were recruited for the study. The institutional animal ethics committee approved the study.

Experimental drugs

Doxorubicin is an anticancer drug (ADRIM) manufactured by Dahur India Ltd. (Pharmaceutical Sector, Solan-173205), India. Rats in the experimental group were given 18 intraperitoneal doses of 1 mg/kg three times a week.[18], group number II. The control group was Group No., to which 0.5 ml/day of physiological saline was administered for a total of 52 days. (Me). Animal weights were monitored at weekly intervals.

Analytical Procedures

Extraction of tissue

The animals were kept in the same lab environment following treatment for two to three days. Before dissection, ether anesthesia was used to sedate the animals. The liver, kidney, and heart were taken after the rats had been killed, and any adhering fat on these tissues was cleaned off and washed with cooled normal saline (0.85%). These were then processed for structural and biochemical tests, including blotting, weighing, and processing.

A 10% homogenate was prepared by placing 0.5 g of tissue in 5 ml 0.1 M sodium phosphate buffer (pH 8.0). The homogenate was centrifuged at 9000 rpm for 20 minutes, and

the resulting supernatant fraction was used for antioxidant enzyme measurements. Reductions in glutathione and lipid peroxidation were estimated using samples treated with 25% and 5% trichloroacetic acid (TCA). The enzymes cytochrome P450 and cytochrome b5 were evaluated from the microsomal fraction obtained after centrifugation at 105,000 xg.

Measurement of malondialdehyde (MDA)

The thiobarbituric acid (TBA) assay was used to assess the concentration of MDA in the 10% homogenates of liver, kidney, and heart (made in 0.1 M sodium phosphate buffer pH 8.0). It is approximated using Esterbauer and Cheeseman's methodology[19].

The total amount of protein

Using the Lowry et al. method, the total protein content in doxorubicin-treated samples was examined and contrasted with controls[20].

Measurement of antioxidants*:* reduced glutathione (GSH)

GSH plays a role in maintaining cellular redox potential in living systems. GSH is according to Moron et al. The developed method was highly regarded[21].

Antioxidant Enzyme Activity: Glutathione Peroxidase (GPx)

GPx (EC 1:11:9) plays an instrumental role in maintaining the redox state during acute oxidative stress. The protective role of GPx is associated with antioxidant enzymes[22].

The supernatant was further used to determine the GPx enzyme by the method of Rotruck et al[23].

Antioxidant Enzyme Activity: Glutathione Reductase (GR)

The activity of GR (EC 1.6.4.2) was estimated by the method of Racker[24].

Antioxidant Enzyme Activity: Catalase (CAT)

CAT (EC 1.11.1.6) activity was measured in accordance with Aebi's instructions[25].

Drug Metabolizing Enzymes' Activities

Antioxidant Enzyme Determination: Glutathione-S-Transferase (G-S-T)

The supernatant was further used for GST (EC 2.5.18) according to Habig's method[26].

Antioxidant Enzymes Determination: Cytochrome P_{450} (CYTp450) and Cytochrome b_5 (CYTb5)

CYTp450 (EC 14.14.1) and CYTb5 (EC 1.6.2.2) were tested in accordance with Omura and Sato's techniques[27].

Structural Studies

Light microscopy

Light microscopy of hepatic and renal tissues was observed and studied by the paraffin method. The liver and kidney were cut into small pieces and fixed in Bouin's fixative[28] for 24-28 hours.

To remove excess Bouin's fixative, the tissues were removed from the fixative and thoroughly washed with distilled water for several hours. After fixation, samples were washed with 70% alcohol to remove excess picric acid from the tissue and dehydrated through a graded series of ethanols. Tissues were decarburized using a series of graded xylene-alcohol mixtures, and the dealcoholized clear tissues were stored overnight at 58–60 °C in filtered molten paraffin. Finally, the tissue was embedded in filtered molten paraffin. Serial 3.0 ìm thick sections of all tissues were obtained using a rotary microtome (Microm, model #HM 310), and sections were stained with hematoxylin followed by a secondary stain, eosin. Slides were mounted using DPX mounting medium as glue, and sections were viewed under a microscope and photographed.

Electron microscopy

The techniques for histological processing of biological material for electron microscopy are basically similar to those used for light microscopy. Compared to light microscopy, the use of electron microscopy gives higher resolution and magnifications.

Transmission electron microscopy

Liver and kidney tissues were removed from rats, cut into 1 mm pieces with a drop of 3% glutaraldehyde, and immersed in fresh, ice-cold fixative for 2 hours and then in 0.1 M cacodylate buffer for 4 hours. Tissues were then briefly rinsed in buffer and post-osmicated with 1% osmic acid for 1–2 hours. The tissues were then dehydrated in a series of increasing alcohols, followed by dehydration in propylene oxide, and finally embedded in polymerized resin at 60 °C. After that, Araldite blocks were prepared, and 1 m sections were cut with a glass knife on the LKB-2000S, an ultramicrotome mounted on glass slides and stained with buffered toluidine blue. Appropriate areas were selected using a light microscope. Finally,

ultra-thin sections of selected blocks were cut with a diamond knife, recorded on copper grids, and stained with uranium acetate and lead citrate for final viewing. Ultrathin sections were scanned and photographed with a JEM-JEOL 100S electron microscope.

Statistics

The data are presented as (mean ± SEM). Statistical analysis was performed using a t-test. Statistically significant at ($p<0.05$).

RESULTS

General observations

The body weights of doxorubicin rats were significantly lower than those of the corresponding controls. Body organs such as liver weights showed non-substantial increases in doxorubicin-treated rats with respect to controls. Kidney weights showed a non-significant decrease in the doxorubicin-treated group in comparison to the control group. After doxorubicin induction, the heart weight had a non-significant decrease compared to controls. Relevant data are collected in (Table 1).

Antioxidant and Antioxidant Enzyme Activity

Eight weeks after doxorubicin injection, GSH activity showed a non-significant increase in liver tissue, but a non-significant decrease in kidney tissue. On the other hand, heart tissue increased significantly. The data are shown in (Tables 2, 3, and 4).

Concentrations of MDA as an index of lipid peroxidation were measured in three organs. Total MDA in liver, kidney, and heart tissue formed in the doxorubicin-treated groups showed a significant decrease compared to the control group (Tables 2, 3, and 4).

The activity of G-S-T was assessed and found to be significantly increased in liver, kidney, and heart tissues treated with the anticancer drug doxorubicin compared to control rats (Tables 2, 3, and 4).

Eight weeks after doxorubicin injection, the activity of GPx showed a significant decrease in liver, kidney, and heart tissues compared to their control counterparts (Tables 2, 3, and 4).

CAT activity in liver tissue was significantly increased in doxorubicin-treated samples, whereas CAT activity in kidney and heart tissue of doxorubicin-treated rats was significantly lower than that of control rats (Tables 2, 3, and 4).

Table 1. Doxorubicin-induced body weight and organ weight changes in male rats (mean ± SEM)

Parameter	Groups	
	Control	Doxorubicin
Body weight (g)	443 ± 4.35	314 ± 4*
Liver weight (g)	11.46 ± 0.654	11.781 ± 0.419#
Kidney weight (g)	1.261 ± 0.045	1.054 ± 0.398#
Heart weight (g)	0.928 ± 0.045	0.884 ± 0.028#

Doxorubicin rats versus matched control rats: *Significant changes from control values are expressed as ($p<0.05$). # ($p<0.05$) represents no significant difference from the corresponding counterparts.

Table 2. Effect of doxorubicin on hepatic antioxidant, antioxidant enzymes, and protein profile of male rats (mean ± SEM)

Parameter	Groups	
	Control	Doxorubicin
Protein(mg/g tissue)	371.068 ± 14.927	406.053 ± 18.615*
LPXn (nmoles of MDA formed /mg protein)	0.20 ± 0.02	0.0162 ± 0.00086*
GSH (µmoles/mg protein)	13.8 ± 1.3	17.095 ± 1.066 #
G-S-T (çmoles of CDNB bound / min /mg protein)	6.3 ± 0.4	9.552 ± 0.275*
GR (çmoles of NADPH red / min/ mg protein)	5.0 ± 0.4	9.32 ± 0.43*
GPx (µg of GSH utilized/ min/mg protein)	269 ± 24.06	27.789 ± 2.595*
CAT (units /mg protein)	0.02 ± 0.00008	0.4322 ± 0.0195*
CYTp450 (nmoles of CO bound /mg protein)	444 ± 37.46	334.889 ± 29.777*
CYTb5 (nmoles of CO bound /mg protein)	615 ± 37.0	837.177 ± 61.197*

Doxorubicin rats versus matched control rats: *Significant changes from control values are expressed as ($p<0.05$). # ($p<0.05$) represents no significant difference from the corresponding counterparts.

The doxorubicin-treated group showed a significant decrease in CYTp450 levels in liver and kidney tissues, and a significant increase in CYTp450 levels in heart tissue compared to control rats (Tables 2, 3, and 4).

Upon treatment with doxorubicin, a significant increase in CYTb5 was observed in liver and heart tissue, whereas levels of CYTb5 in kidney tissue were significantly decreased compared to their control counterparts (Tables 2, 3, and 4).

For doxorubicin, total protein in liver and kidney tissues showed a significant increase, whereas total protein content in the heart fraction did not show a significant decrease compared to the control group (Tables 2, 3, and 4).

Histological effects of doxorubicin on hepatic tissue

In the present study, it was evident that the liver cells displayed few distinct changes as a result of treatment with doxorubicin, detected in the tissues by light microscopy (Figures 1 and 2).

Electron microscopy data obtained from this study showed that after doxorubicin induction, rats exhibited slight structural changes, numerous mitochondria, continuity between rough and smooth endoplasmic reticulum, the presence of lipid droplets of different densities, and very strong evidence of microvilli. A long sine wave marked the room. Most organelles are visible (Figures 5, 6, 7, and 8).

Table 3. Effect of doxorubicin on renal antioxidant, antioxidant enzymes, and protein profile of male rats (mean ± SEM)

Parameter	Groups	
	Control	Doxorubicin
Protein (mg/g tissue)	262.736 ± 17.356	356.206 ± 21.959*
LPXn (nmoles of MDA formed /mg protein)	0.31 ± 0.03	0.0239 ± 0.0003*
GSH (µmoles/mg protein)	8.06 ± 0.74	7.9462 ± 0.781#
G-S-T (çmoles of CDNB bound / min /mg protein)	1.46 ± 0.11	6.4261 ± 0.101*
GR (çmoles of NADPH red / min/ mg protein)	9.98 ± 0.37	15.3682 ± 0.44*
GPx (µg of GSH utilized/ min/mg protein)	415.4 ± 25.14	98.8393 ± 1.571*
CAT (units /mg protein)	0.6 17 ± 0.09	0.46237 ± 0.019*
CYTp450 (nmoles of CO bound /mg protein)	683.4 ± 40.5	288.762 ± 25.806*
CYTb5 (nmoles of CO bound /mg protein)	2605.5 ± 259.2	447.685 ± 35.215*

Doxorubicin rats versus matched control rats: *Significant changes from control values are expressed as ($p<0.05$). # ($p<0.05$) represents no significant difference from the corresponding counterparts

Table 4. Effect of doxorubicin on cardiac antioxidant, antioxidant enzymes, and protein profile of male rats (mean ± SEM)

Parameter	Groups	
	Control	Doxorubicin
Protein (mg/g tissue)	334.26 ± 13.129	326.69 ± 16.266#
LPXn (nmoles of MDA formed /mg protein)	47.451 ± 1.708	0.0398 ± 0.00097*
GSH (µmoles/mg protein)	3.32 ± 0.308	4.786 ± 0.478*
G-S-T (çmoles of CDNB bound / min /mg protein)	1.715 ± 0.158	3.452 ± 0.086*
GR (çmoles of NADPH red / min/ mg protein)	0.877 ± 0.051	2.254 ± 0.181*
GPx (µg of GSH utilized/ min/mg protein)	379.794 ± 33.345	137.722 ± 13.387*
CAT (units /mg protein)	7.277 ± 0.331	0.565 ±0.016*
CYTp450 (nmoles of CO bound /mg protein)	25 ± 0.24	312.31 ± 24.261*
CYTb5 (nmoles of CO bound /mg protein)	88 ± 0.4	165.352 ± 8.7*

Doxorubicin rats versus matched control rats: *Significant changes from control values are expressed as ($p<0.05$). # ($p<0.05$) represents no significant difference from the corresponding counterparts.

Doxorubicin-treated rat liver under a light microscope

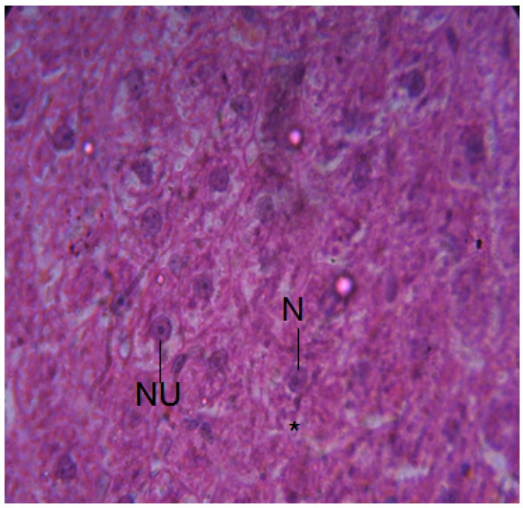

Fig. 1. Light micrographs of rat livers treated with doxorubicin for 8 weeks show the presence of vacuolated hepatocytes (*). It also shows the parenchyma (p) and many vacuoles around the nucleus. Prominent nucleoli (NU) in almost all nuclei are also seen. Photographed at a magnification (x 40).

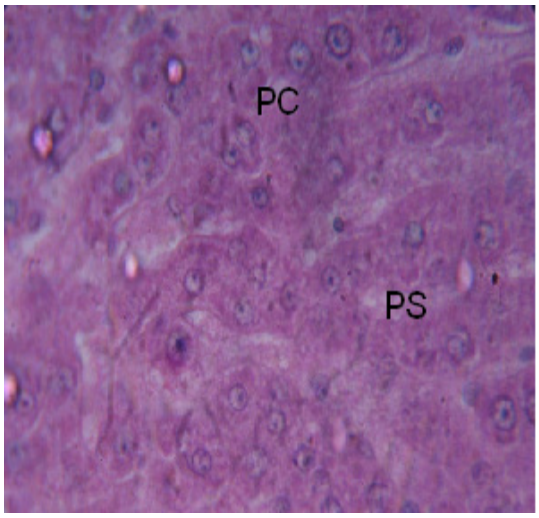

Fig. 2. A light micrograph of a rat liver treated with doxorubicin for 8 weeks shows a narrow portal space (PS). Vacuoles can be seen. A dangerous connection is observed. Photographed at magnification (x 40).

Doxorubicin-treated rat kidneys under a light microscope

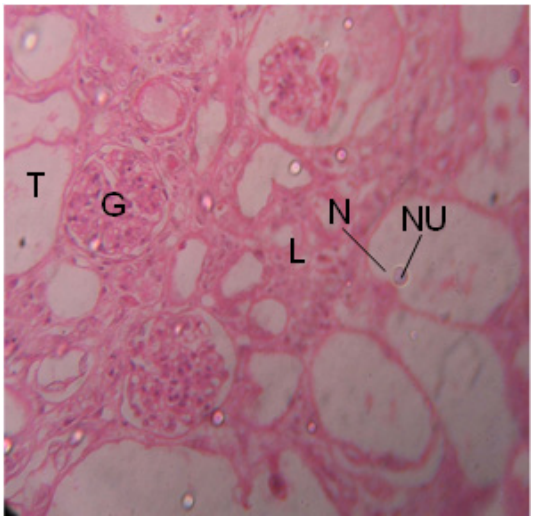

Fig. 3. Light micrographs of rat kidneys treated with doxorubicin for 8 weeks show subtle changes in the tubules (T). Glomeruli (G) were observed. Lumen (L) and vacuolization (*) are observed. Photographed at magnification (x 40).

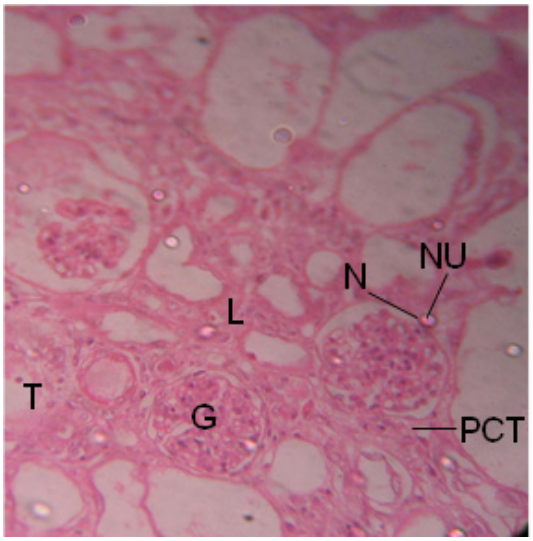

Fig. 4. Light micrographs of rat kidneys treated with doxorubicin for 8 weeks show dilated proximal tubules (PCT). Nuclei (N) and prominent nuclei (NU) can be seen. Note the lipid droplets in some tubules. Photographed at magnification (x 40).

Doxorubicin-treated rat liver photographed under an electron microscope

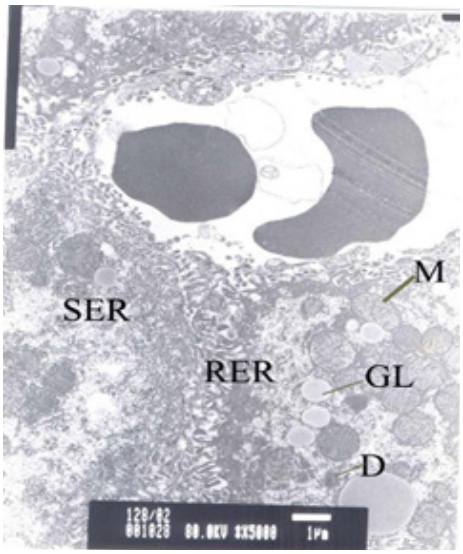

Fig. 5. Electron micrographs of rat livers treated with doxorubicin for 8 weeks show that hepatocytes contain many organelles. Many intact mitochondria were observed. Note the cytoplasm with numerous sequences of rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). Desmosomes (D) were also seen. Glycogen (Gl) is also shown. Image magnification (x5,000).

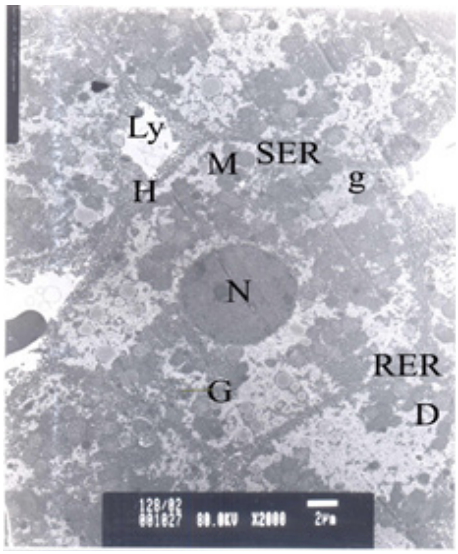

Fig. 6. Electron micrographs of livers from rats treated with doxorubicin for 8 weeks clearly show the nucleus (N) near the center of the figure, and the cytoplasm consists of smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), as well as mitochondria (M). Lysosomes (Lys) are seen. Desmosomes (D) and secretory granules (G) are also present in large regions of heteroaromatic boundaries (H). Photographed at magnification (x 2,000).

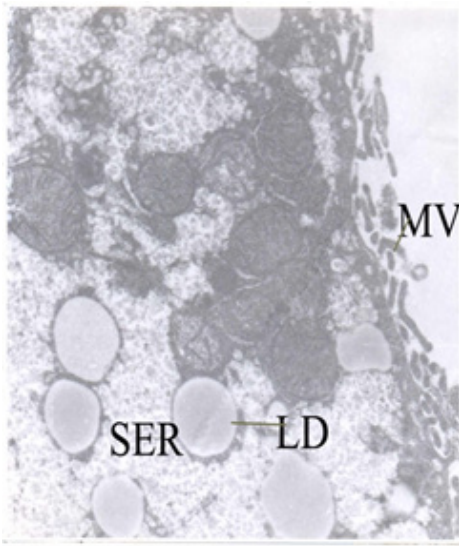

Fig. 7. Electron micrographs of rat livers treated with doxorubicin for 8 weeks show that mitochondria (M) are present throughout the cytoplasm, with a smooth endoplasmic reticulum (SER). Lipid droplets (LD) were also found in the cytoplasm. Short and irregular secretory granules (G) and microvilli (Mv) are present. Photographed at magnification (x 15,000).

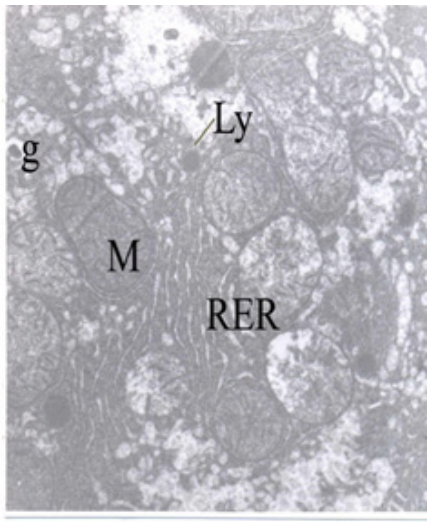

Fig. 8. Electron micrographs of rat livers treated with doxorubicin for 8 weeks show cytoplasmic accumulation of large mitochondria (M). Lysosomes were also recognized. Note the presence of an abundant array of roughened endometrium (RER). Photographed at a magnification (x 15,000).

Doxorubicin-treated rat kidney in electron micrograph form

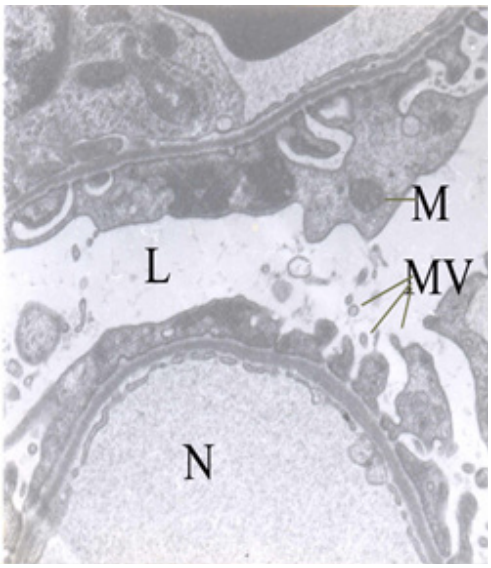

Fig. 9. Electron micrographs of rat kidneys treated with doxorubicin for 8 weeks. Electron micrographs of several lysosomes (Ly) were visible and the lumen (L) was also evident. Note the low number of mitochondria (M) with a dense electronic matrix. Image magnification (x15,000).

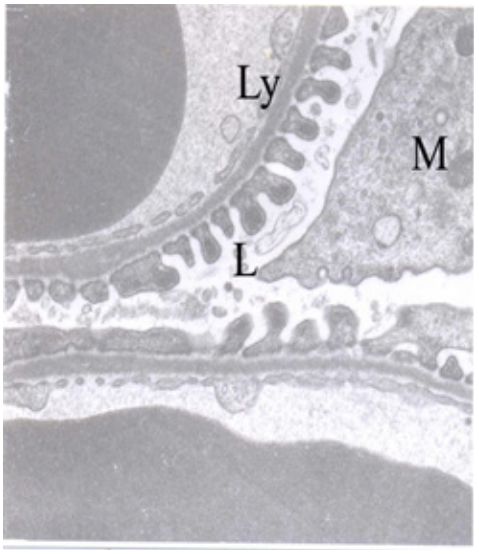

Fig. 10. Electron micrographs of rat kidneys treated with doxorubicin for 8 weeks. Electron micrographs of epithelial microvilli (Mv) are clearly visible. Some sections of the lumen are shown. The mitochondria are present and the nucleus is also visible. Photographed at a magnification (x 20,000).

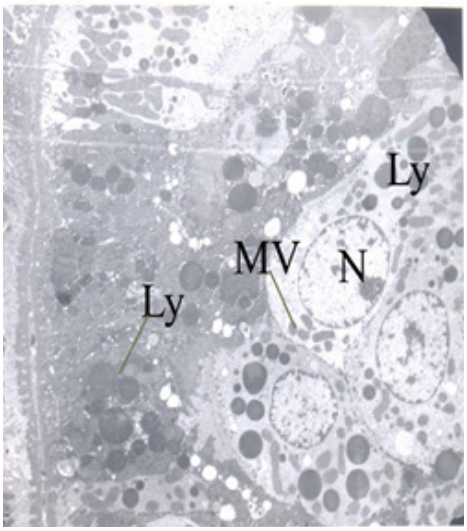

Fig. 11. Electron micrographs of rat kidneys treated with doxorubicin for 8 weeks. Electron micrographs of nuclei (N) are present, microvilli (Mv) are evident, and lysosomes (Ly) are visible. Image magnification (x3,000).

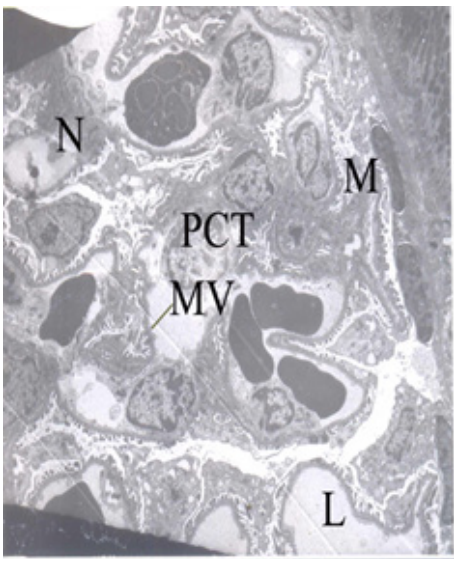

Fig. 12. Electron micrographs of rat kidneys treated with doxorubicin for 8 weeks. Electron micrographs of less elaborately shaped cells and mitochondria (M) have a more circular profile than subsequent complex sections. Image magnification (x3,000).

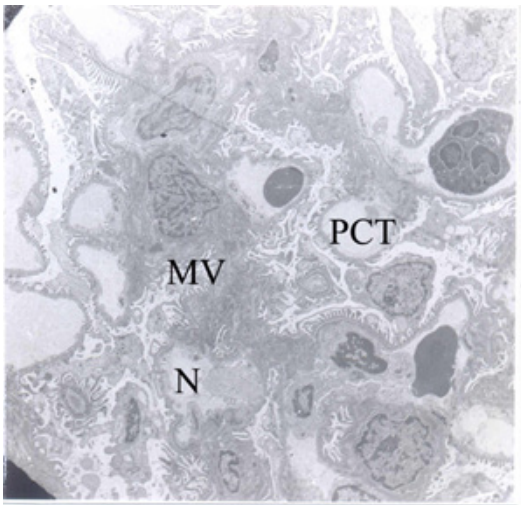

Fig. 13. Electron micrographs of rat kidneys treated with doxorubicin for 8 weeks. Note the presence of epithelial microvilli (MV). The proximal tubule (PCT) is a prominent core of tubular lining that shows dense chromatin. Photographed at magnification (x 2,500).

Histological effects of doxorubicin on renal tissue

Eight weeks after doxorubicin treatment, kidney sections were examined under a light microscope and evaluated blindly. The renal cortex was nearly normal in appearance, and the glomeruli and glomerular quadrants showed non-significant changes in renal-treated rats. This implies that the production of toxic oxygen is one of the mechanisms leading to kidney tissue damage, which is not seen in this case (Figures 3 and 4).

Electron micrographs show little change in the histological aspect of the lumen, with fewer recognizable microvilli, fewer lysosomes, and recognizable mitochondria. The core appears very clearly and in large quantities. Also of note is the proximal convoluted tube. This is seen in the doxorubicin model (Figures 9, 10, 11, 12, and 13).

DISCUSSION

The rats' body weight was checked weekly. Our results are consistent with those of Adachi et al.[29], who reported significant weight loss in rats after treatment with doxorubicin. The data obtained in our study are also consistent with those published by Imahie et al. and Manabe et al.[30, 31], who reported a significant decrease in body weight after doxorubicin injection in rats.

Our study shows that doxorubicin treatment in rats resulted in significant weight loss due to anorexia and accelerated doxorubicin utilization. Some toxicity may be associated with doxorubicin at high doses.

In this study, organ weights were determined. Daikoku et al.[32,33] reported that doxorubicin adversely affected hematopoietic organs, including the liver and kidney, which is consistent with our study. In a previous study, Boraman et al.[34] reported a non-significant decrease in heart weight in rats following doxorubicin induction and pretreatment with amifostine compared to controls (mouse or rat).

GSH is involved in the protection of cellular redox potential against oxidative stress in biological systems. It is also known to be involved in maintaining normal kidney structure and function (Anand et al.[35].

According to Zaman et al.[36], the effectiveness of medications like doxorubicin and cisplatin in cancer cell lines is decreased by the reduction in intracellular GSH levels caused by its oxidation by ROS. Previous studies have shown that doxorubicin sensitivity increases and free radical damage to cells increases when cellular reduced glutathione levels are low[37].

According to reports, reactive oxygen intermediates (ROI) are crucial to doxorubicin toxicity. Increased free radical generation, reduced ROI breakdown by antioxidant enzymes (AOE), or a combination of the two can all contribute to increased ROI concentration under pathological conditions. An increase in production has the power to start lipid peroxidation[38].

Lipid peroxidation is an influential cardiotoxicity index of doxorubicin, especially under conditions of acute exposure. Doxorubicin was found to be more selective in inducing this oxidative damage in the heart compared to other organs such as the liver in mice[39]. Cisplatin and transplatin decreased lipid peroxidation in the liver, kidney, and platelets[40,41]. Our study showed that the concentration of MDA as an indicator of lipid peroxidation in liver, kidney, and heart tissues showed a significant decrease compared with the control group. Possibly due to the low levels of

free radicals produced by this drug or in these tissues mentioned above, cell membranes are less susceptible to oxidative damage by free radicals.

Pre-irradiation treatment with P. headroom improved liver G-S-T, according to Mittal et al.[43]. According to Awasthi et al.,[44], GSH creates a complex molecule with doxorubicin in the presence of a G-S-T catalyst to create a drug conjugate that is less hazardous than the parent chemical. The data from our investigations are consistent with those from two prior studies.

A key role of G-S-T is the detoxification of endogenous products of lipid peroxidation, such as 4-hydroxy-2-nonenal. Human G-S-TA 4-4 has very high activity towards this substrate and is critical in physiology for protecting against oxidative stress induced by endogenous lipid peroxidation. It is possible that will play a role[45].

Shepherd et al.[46] noted that glutathione reductase activity increased in liver tissue, which is consistent with our report of a group of liver, kidney, and heart tissues that were treated with doxorubicin. Increased levels of free radicals or a metabolite of an anticancer medication may cause enzyme inactivation.

Antioxidant enzyme (GPx) parameters serve as one of the indicators of oxidative stress on tissues after doxorubicin treatment in rats. The GPx reduction of the anticancer drug doxorubicin plays a protective role, consistent with previous reports by Staherin et al.[22]. I agree.

Catalase levels in liver tissue were increased in models of the anticancer drug doxorubicin, suggesting quenching of hydrogen peroxide. The findings of our study are consistent with those of the authors Yin et al.[47]. Presumably, this attack by free radicals and reactive oxygen species on hepatic doxorubicin was potent enough to enhance the activity of catalase. Mean CAT concentrations are already significantly reduced in kidney and heart tissue. Branden et al.[38] concluded that after 10 weeks of doxorubicin treatment in rats, CAT levels were significantly reduced, supporting our results.

According to Bachur et al.[48], the enzyme CYTp450 is in charge of doxorubicin's one-electron reduction into a semi-quinone free radical.

It's possible that reduced CYTp450 levels have slowed down the metabolism of the medication doxorubicin. In the instance of rats treated with hepatic and renal tissues, our result agrees with Nebert and Russell's[49] findings the elevated levels of cytochrome p450 in the cardiac tissue of the doxorubicin-treated groups may be the result of an increase in both the substrate and their metabolism.

Nebert and Russell[49] reported that CYTp450 and b5 play critical roles in the process of xenobiotic biotransformation and bioactivation. There is a possibility that cytochrome b5 depletion indicates the involvement of ROS in renal doxorubicin-induced enzyme inactivation, while cytochrome b5 may be involved in liver and heart cases. This is due to the strong interactions between this enzyme and different CYT-p450 species.

The cell's overall protein patterns vary in response to medication therapy. These changes in protein levels may be caused by drug-induced denaturation and degradation of existing proteins or by the inducible synthesis of newly synthesized or existing proteins.

In the current study, it is evident that doxorubicin treatment causes small, visible alterations in the liver cells that can be seen when the tissues are microscopically examined. There are several freely available experimental models. Zicca et al.[50] showed that cisplatin-induced rat parenchyma exhibits structural alterations, which leads to hepatic injury, which contrasts with our findings. After administering CCl4 to rats, Bridges et al.[51] observed that altered liver function occurred. According to recent studies, doxorubicin therapy causes minor liver damage. After receiving doxorubicin treatment, the renal cortex displayed an almost normal appearance. In contrast, the glomeruli and glomeruli quadrants showed little change in the kidney. In a case of 10 weeks of doxorubicin treatment, Branden et al.'s[38] findings matched those of our doxorubicin model.

At the level of light microscopy, long-term doxorubicin therapy does not result in any appreciable modifications, while at the level of electron microscopy, histological points of view show little change in histological, ultrastructural, and biochemical characteristics.

In summary, our data demonstrate that in the doxorubicin model, anticancer drug-induced organelle changes are observed by light and electron microscopy. Enzymatic and non-enzymatic changes were examined in the liver,

kidney, and heart using doxorubicin, compared to controls, and highlighted.

The current study aims primarily to understand the antioxidant architecture of commonly used anticancer drugs and their toxic effects on the detoxification system in experimental rat models. Finally, we examined the effects of anticancer agents such as doxorubicin on albino rats.

CONCLUSION

According to the findings of the current study, the anticancer drug Doxorubicin (Adriamycin) has a significant influence on rats used as an animal model, and these findings are crucial for understanding the mechanism of toxicity on many organs like the liver, kidney, and heart.

Free radicals cause tissue oxidative stress, which can be prevented by altering enzyme and antioxidant levels. This research illustrates the effectiveness of a key chemotherapeutic drug in the management of mammalian cancer.

A combination of doxorubicin and etoposide along with cisplatin, 4-hydroxyperoxycyclophamide, and vindesine has been reported to successfully kill tumors with synergistic antitumor activity in vitro[52]. According to our results, it can be assumed that the combination of these drugs, doxorubicin and etoposide, or other drugs such as cisplatin, can improve activity and minimize side effects.

ACKNOWLEDGMENT

I would like to thank Princess Tharwat University College, affiliated with Balqa Applied University, Muhawish Yusuf, Amman, Jordan, for providing help, assistance, and support throughout my research. We thank the Dean and Vice Dean of Princess Tharwat University College, affiliated with Balqa Applied University, Muhawish Yusuf, Amman, Jordan, for making available research, and development facilities to undertake the present work. And technical support with regard to the computation of the data is gratefully acknowledged.

Conflict of Interest

The author declares that there are no conflicts of interest relevant to this article.

Funding Source

There is no fund was received.

REFERENCES

1. Di Marco, A., Gaetani, M. Scarpiato, B., 1969. Adriamycin (NSC-123127), a new antibiotic with anti-tumor activity. Cancer Chemother Rep. 53, 33.37.
2. Barpe DR, Rosa DD, Froehlich PE. 2010 Pharmacokinetic evaluation of doxorubicin plasma levels in normal and overweight patients with breast cancer and simulation of dose adjustment by different indexes of body mass. Eur J Pharm Sci. 41(3–4):458–463.
3. Mitry MA, Edwards JG. 2016. Doxorubicin-induced heart failure: phenotype and molecular mechanisms. IJC Heart Vasc.; 10:17–24.
4. Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, et al. 1969. Adriamycin, 14-hydroxydaunomycin: a new antitumor antibiotic from S. peucetius var. Caesius. Biotechnol Bioeng.11,1101–1110.
5. Weiss RB. 1992. The anthracyclines: will we ever find a better doxorubicin? Semin Oncol.; 19:670–686.
6. Brown, H., 1963. Anthracycline Und. Fostschr. Chem. Org. Naturstoffe 21, 121 – 182.
7. Brown, J.R., 1978. Adrimycin related anthraycline antibiotics. Prog. Mod. Chem.15, 125 – 164.
8. Andringa, K.K., 2. April. 2001. Adriamycin: As Good as it gets Free Radical and Radiation Biology Program.99, 391-2.
9. Katzung BG, Masters SB, Trevor AJ. 2012. Basic & Clinical Pharmacology. 12th ed. McGraw Hill.
10. Schaupp CM, White CC, Merrill GF, Kavanagh TJ. 2015. Metabolism of doxorubicin to the cardiotoxic metabolite doxorubicinol is increased in a mouse model of chronic glutathione deficiency: a potential role for carbonyl reductase 3. Chem Biol Interact.; 234:154–161.
11. Luu AZ, Chowdhury B, Al-Omran M, Teoh H, Hess DA, Verma S. 2018. Role of the endothelium in doxorubicin-induced cardiomyopathy. JACC Basic to Transl Sci.;(6):861–870. Doi: 10.1016/j.jacbts.
12. Sakai-Kato K, Saito E, Ishikura K, Kawanishi T. 2010. Analysis of intracellular doxorubicin and its metabolites by ultra-high-performance liquid chromatography. J Chromatogr B Anal Technol Biomed Life Sci.;878(19):1466–1470.
13. Powis, G 1989. Free radical formation by antitumor quinines. Free Rad. Biol. Med. 6, 63.
14. Halliwell, B., Guteridge, J.M.C. 1989. Free Radicals in Biology and Medicine, 2nd Ed.,

Clarendon Press, Oxford.

15. Halliwell, B., Cross, C.E. 1991. Reactive oxygen species, antioxidants and acquired immunoficiency syndrome. Arch. Int. Med.157:29-32;5.
16. Trush, M.A, Kensler, T.W., 1991. Role of free radicals in carcinogen activation. In: Sics H, cd. Oxidative stress: oxidants and antioxidants. Academic Press, London: 277-318.
17. Liu L.F., Liu C, C., Albert B.M. 1980. Type II DNA topoisomerases: enzymes that unknot a topologically knotted DNA molecule via a reversible double-stranded break. Cell 19,697-707.
18. Ward J.A., Bardin C.W., Knight M., Robinson J., Gunsalus G. and Morris I.D. 1988. Delayed effects of doxorubicin on spermatogenesis and endocrine function in rats. Reprod. Toxical. 2,117-126.
19. Esterbauer, H., Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynoneneal. Methods Enzymol. 186, 407– 414.
20. Lowry, O.H., Rosebrough, N.J., Farr, A.L Randall, R.J., 1951. Protein measurements with the Folin Phenol Reagent. J. Biol. Chem. 193, 265-274.
21. Moron, M.S., Joseph, W.D., Mannervik, B., 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim. Biophys. Acta 582, 67–78.
22. Stahelin, H.F., Von Wartburg, A., 1991. The chemical and biological route from podophyllotoxin glucoside to etoposide: The Ninth Cain memorial award lecture. Cancer Res. 51, 5-15.
23. Rotruck, J.T., Pope, A.L., Ganther, H.A., Wanson, A.B., Hafeman, D.G., Hoeckstra, W.G., 1973. Selenium-a biochemical role as a component of glutathione peroxidase. Science 179, 588–590.
24. Racker, E. 1955. Glutathione reductase from baker's yeast and beef liver. Eur. J. Biol. Chem. 217, 855–864.
25. Aebi, H., 1984. Catalase in vitro. Methods of Enzymol. 105, 121–126.
26. Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 240, 7130–7139.
27. Omura, T., Sato, R., 1964. The carbon monoxide-binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. J. Biol. Chem. 239, 2370–2378.
28. Humason, Gretchen, L., 1979. Animal tissue techniques, W H Freemen & CO., San Francisco.
30. Adachi T, Nishimura T, Imahie H, Yamamura T. 2000. Collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rats. Testicular toxicity in male rats given Adriamycin for two or four weeks. J Toxicol Sci. 25,95–101.
30. Imahie, H., Aachi, T., Nakagawa, Y., Nagazaki, T., Yamamura, T., Hori M., 1995. Effects of Adriamycin, an anticancer drug showing testicular toxicity, on fertility in male rats. J. Toixal. Sci. 20,183-193.
31. Manabe, Fumino, Takeshima, H., Takeshima, H., Akaza, H., 1996. Protecting spermatogenesis from damage induced by doxorubicin against leuprorelin. Am. Cancer Soc. 4, 1014-1021.
32. Oguro, T., Miyazaki, E., Hara, T., Hataro M., Shimizu, M., 1973a. A study on the safety of Adriamycin HCL. Report No.2: Subacute toxicity study. Clin. Rep. (Kiso to Rinsho), 7, 1063-1103.
33. Oguro, T., Miyazaki, E., Hara T., Hataro, M., Shimizu, M., 1973b A study on the safety of Adriamycin HCL. Report No.3: Chronic toxicity study. Med. Treat. (Yakubutsu Ryoho), 6, 1093-1151.
34. Bolaman, Z., Cicek C., Dikoylu, G., Serter M., Alper, G., 2005. The protective effects of aminostine on Adriamycin –induced acute cardiotoxicity in rats. Tohoku J.Exp. Med.. 207,249-253.
35. Anand, C.V., Anand, U., Agarwal, R., 1995. Antioxidant enzymes, gamma-glutamyl transpeptidase and lipid peroxidation in the kidneys of rats exposed to cigarette smoke. Indian J of Exp Bio.34, 486-488.
36. Zaman G.J., Lankelma J., Van Tellingen O., Beijnen J., Dekker H., Paulusma C., Oude Elferink R.P., Baas F., Borst P., 1995. Role of glutathione in the export of compounds from cells by the multidrug resistance associated proteinProc. Natl. Acad. Sci-USA.A. 92, 7690-7694.
37. Taylor, Davenport LD, Speramza M J, Mullenbach GT, Lynch RE., 1993. Glutathione peroxidase protects cultured mammalian cells from the toxicity of adrimycin and paraquat. Arch Bioch Biophys. 305(2), 600-5.
38. Branden, C.V., Ceyssen, B.D., Craemer, D., Peuwels, D., Peuwels, M., Houte K.V.2000. Renal antioxidant enzymes and fibrosis-related markers in the adriamycin model. Nephron 86,167-175.
39. Kang, S., James, Y., ChenYan, Epstein, N., Paul. 1996. Suppression of Doxorubicin Cardiotoxicity by Overexpansion of Catalase in the Heart of Transgenic Mice. JBC on line volume 271, 12610-12616.
40. Sadzuka Y., Shoji T., Tukino Y., 1991. Change in

lipid peroxide levels in rat tissue after cisplatin administration. Toxical. Lett. 57,159-166.

41. Olas B, Wachowicz B., 1996. Cisplatin-induced changes in the biological activity of blood platelets: Thiol-related mechanisms. Anticancer drugs 7(4), 476-482.
42. Bompart, G.J., Prevot, D., and Jean-Loup, B. (1990) Rapid automated analysis of glutathione reductase, peroxidase, and S-transferase activity: application to cisplatin-induced toxicity. Clini. Biochem 23, 501-504.
43. Mittal, A., Pathania, V., Agrawala P.K., Prasad, J., Singh, S., Goel, H.C., 2001. Influence of podophylum hexandrum on the endogenous antioxidant defence system in mice: Possible role in radioprotection. J. Ethnophamacol. 76, 253-62.
44. Awasthi, Yogesh, C., Sanjay, Awasthi., Piotr, Zimniak., 1997. Multiple transport proteins are involved in the detoxification of endo- and xenobiotics. Frontiers of the Biosciences. 2, d 427-437.
45. Hubastsch, I., Ridderstrom, M., Mannervik, B., 1998.Human glutathione transferase A4-4: An alpha class enzyme with high catalytic efficiency in the conjugation of 4-hydroxynonenol and other genotioxics of peroxidation, Biochem.J.330, 175-179.
46. Shepherd A.G., Mauson M.M., Helen W.L. Ball and Mclellan L.I., 2000. Regulation of rat glutamate–cysteine ligase (gamma-glutamylcysteine synthetase) subunits by chemopreventive agents and in aflatoxin B1-induced preneopasia. Carcinogenesis, 21(10), 1827-1834.
47. Yin, X., Wu, H., Chen Kang, Y.J. 1998. Induction of antioxidants by Adriamycin in mouse heart. Chem Biol Interact. 111-112, 333-42.
48. Bachur, N.R., Gordon, S.L., Gee M.V., Kon, H., 1979. NADPH cytochrome P450 reductase activation of anticancer agents to free radicals. Pro. Natl. Acad. Sci. USA. 76,954-957.
49. Nebert, D.W., Russell, D.W., 2002. Clinical importance of cytochrome P450. Lancet 360, 1155–1162.
50. Zicca, A., Cafaggi, S., Mariggio, M.A., Vannozzi, M.O., Ottne, M., Bucchini, V., Caviglioli, G., Viale, M., 2002. Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats. Eur. J. Pharmacol. 442, 265–272.
51. Bridges, J.W., Benford I.M., Hubbard, S.A., 1983. Mechanisms of toxic injury. Annals of the New York Academy of Sciences, 42-61.
52. Henwood,J.M., & Brogden,R.N. 1990. Drug evaluation. Etoposide. Drugs 39:438-490.