Ind. J. Physiol. Pharmac., 1990; 34(2): 94-100

α-TOCOPHEROL REDUCES DOXORUBICIN-INDUCED TOXICITY IN RATS - HISTOLOGICAL AND BIOCHEMICAL EVIDENCES

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(Received on January 17, 1990)

Abstract : The beneficial effect of α-tocopherol on doxorubicin-induced toxicity was studied in rats. α-Tocopherol (400 mg/kg/day) was administered orally, daily for a period of 2 months along with/without doxorubicin (2.5 mg/kg, i v weekly once for 8 weeks). Histology showed liver necrosis, heart myocyte degeneration, glomerular and tubular degeneration, cellular infiltration and desquammation of intestinal mucosa in doxorubicin treated animals. There was a significant increase in lipid peroxide levels measured in terms of "TBA reactants" in all these organs. These changes were associated with elevated levels of serum enzymes such as transaminases, creatine kinase and lactate dehydrogenase. The pathological observations, were minimal in animals receiving both doxorubicin and α-tocopherol. The lipid peroxide levels were low with concomitant normal levels of serum and intestinal enzymes in those animals.

Key words : doxorubicin-lipid peroxidation-tissue necrosis α-tocopherol-antioxidant

INTRODUCTION

The anthracycline antobiotic doxorubicin is one of the potent drugs in the field of cancer chemotherapy (1). However its repetitive administration in patients and in experimental animals has been associated with the development of cardiotoxicity (2) and other drastic side effects (3).

Doxorubicin is known to generate superoxide radical ions either enzymatically (4) or non-enzymatically (5) and to stimulate lipid peroxidation (6). The formation of free radicals as well as accumulation of lipid peroxides in doxorubicin treatment has been well documented, and this is recognised as one of the possible biochemical mechanisms for the doxorubicin associated side effects (6).

Yamanaka et al (7) observed the beneficial effects of antioxidants for protection against doxorubicin-toxicity. Infact, α-tocopherol was shown to prevent cardiotoxicity effectively, presumbly by inhibiting lipid peroxidation.

Since lipid peroxidation has been reported to be associated with various deleterious effects including tissue damage and necrosis, direct evidence like histopathology of a particular organ may throw more light on the effect of α-tocopherol on the doxorubicin-induced lipid peroxidation.

Hence in the present investigation histochemical observations were made on liver, heart, kidney and intestine of doxorubicin treated animals and compared with those coadministered with α-tocopherol, Levels of some clinically important enzymes in serum and in intestinal mucosa were determined and compared.

METHODS

Doxorubicin hydrochloride (Sigma Chemical Company, USA) was dissoved in the vials with sterile saline and used within 48 hr. The solution was kept in ice in a dark atmosphere until use.

Adult male Wistar rats weighing 150-160 g

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were used for the study. The rats were fed with commercial pelleted rat chow and water given *ad libitum*. The rats were divided into 4 groups. Groups 1 served as control. Group 2 rats were injected doxorubicin 2.5 mg/kg, weekly once for a period of 8 weeks (8). Group 3 rats were fed orally, α-tocopherol, 400 mg/kg, daily for 2 months. Group 4 animals received both doxorubicin and α-tocopherol at the above mentioned dosages.

After the experimental period, the rats were killed by cervical decapitation. Blood was collected and the serum separated was used for the assay of transaminases (9) lactate dehydrogenase (9) and creatine kinase (10).

Immediately after the sacrifice, liver, kidney, heart and intestine were removed and washed in ice-cold saline. A portion of the tissues were fixed in 10% formalin-saline and stained with hematoxylin and eosin for histological examinations. Another portion of the tissues were homogenised in 0.1 M phosphate buffer pH 7.4 and used for the estimation of lipid peroxides in terms of "TBA reactants" (11). 1,1'3,3' tetramethoxy propane was used as the standard.

After wasing the food contaminants in the intestine the mucosal scrappings were collected, weighed and homogenised. The homogenate was used for the assay of Na^+, K^+ - ATPase (12) Ca^{2+} ATPase (13) and alkaline phosphatase (14) activities. Protein was determined by the method of Lowry et al (15).

RESULTS

Figs. 1 and 2 show the tissue architecture of liver and heart (a, b and c denote the groups 1, 2 and 4, respectively). Hyperaemia, mild foci of centrilobular necrosis, irregular nuclear pyknosis, diffuse cloudy swelling, mild hydropic changes and focal fatty changes are common in doxorubicin treated rat liver. Fig. 1b shows the region of extensive necrosis. These changes are minimum in group 4 rats. Other changes like cellular infiltration and zones of necrosis observed in doxorubicin treatment are also absent.

Heart sections show focal myocyte degeneration, cloudy swelling, loss of striations and multifocal hemorrhage in doxorubicin treated rats. Proliferation of Anitskor cells and edema were also present. Neither necrosis nor myocyte degeneration are seen in group 4 rats. But hyperaemia and mild hemorrhage are noted.

Glomerular hyperaemia, atrophy of glomerulus with tubular necrosis are frequent in sections of kidney from doxorubicin treated animals (Fig.3)

Intestinal sections (Fig 4) from group 2 rats show necrosis and desquammation of the mucosa and infiltration of mononuclear cells. The goblet cell activity is much reduced. These changes are minimum in group 4 rats.

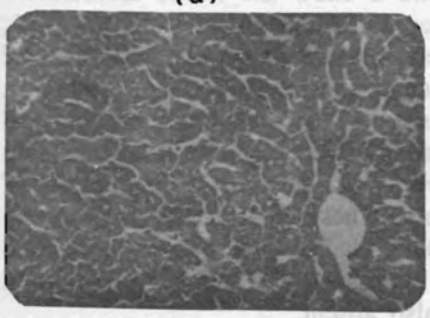
In all these sections control animals show normal architecture and there were no significant

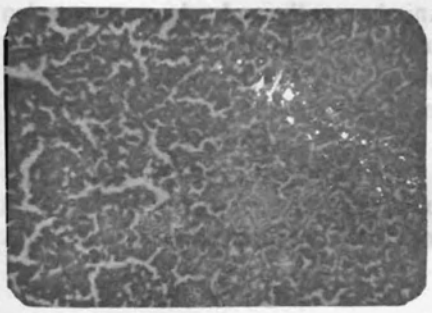
TABLE I : Levels of lipid peroxides in liver, heart, kidney and intestinal mucosa of control and experimental rats. Values are expressed as mean ± SD for 6 rats in each group.

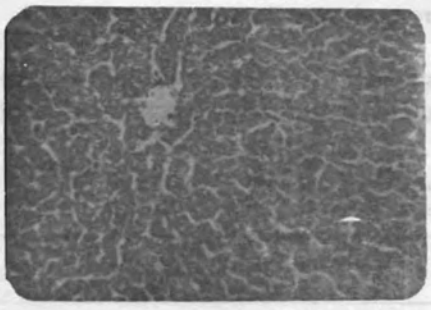
Group	*Rats treated with*	*Liver*	*Heart*	*kidney*	*Intestinal mucosa*
1	None	240.5±12.9	160.2±10.9	262.5±13.6	179.5±11.7
2	Doxorubicin	320.1±19.1**	241.0±11.6**	310.2±11.0**	249.5±20.3**
3	α-tocopherol	230.2±11.6^{NS}	153.0±11.5^{NS}	250.0±20.2^{NS}	170.8±10.8^{NS}
4	Doxorubicin + α-tocopherol	260.3±10.6^{NS}	17120±11.3^{NS}	276.0±12.5^{NS}	190.6±18.5*

Lipid peroxide lelvels of expressed as n moles malonaldehyde/gm tissue.
For statistical analysis Groups 2, 3 and 4 are compared with group
** P < 0.001 ** P < 0.01 NS - Non significant

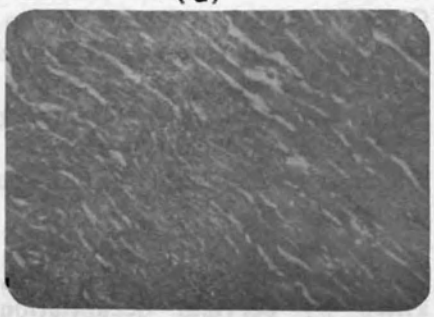
LIVER
FIG·1

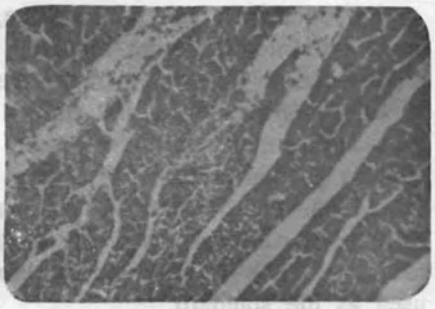






HEART
FIG·2





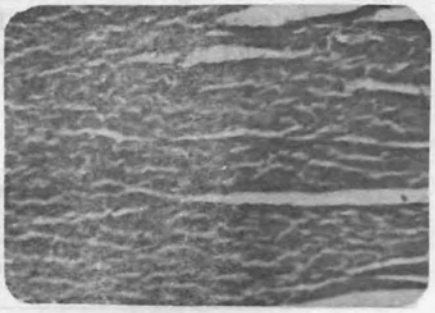
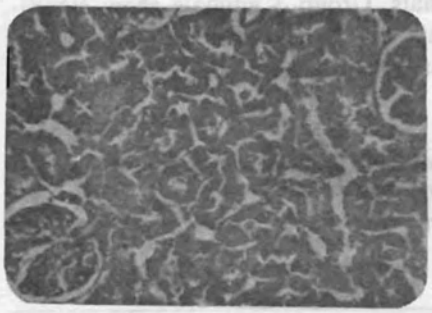


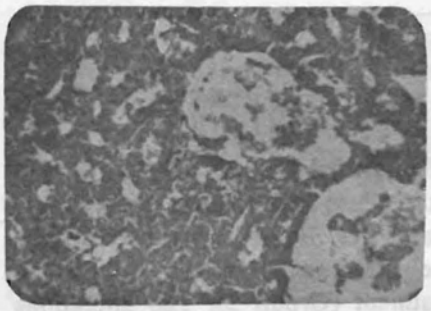
Fig. 1 and 2 :
Histology of liver (Fig. 1) and heart (Fig.2) of rats (Hematoxylin-Eosin x 200).
(a) untreated, control animals; (b) animals treated with doxorubicin and (c) animals treated with doxorubicin + α-tocopherol (Treatment as given in 'Materials and Methods')

Note: Necrosis (1b) and only occasional pyknosis (1c); degeneration of myocytes and marked hemorrhages (2b) and only mild hyperaemia and hamorrhage (2c).

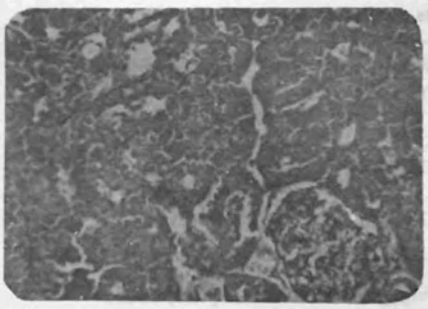
KIDNEY
FIG.3
(a)



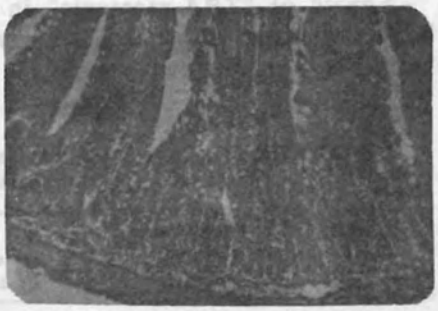
(b)



(c)



INTESTINE
FIG.4
(a)



(b)

(c)

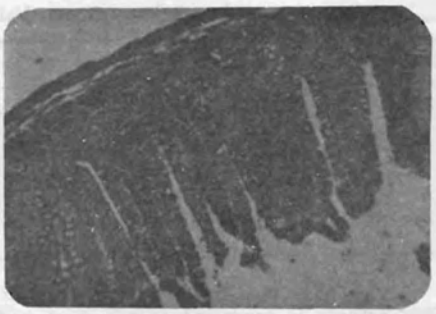


Fig. 3 and 4 :
Histology of kidney (Fig.3) and Intestine (Fig.4) of rats (Hematoxylin-Eosin x 200).
(a) untreated, control animals; (b) animals treated with doxorubicin and (c) animals treated with doxorubicin + α-tocopherol.

Note: glomerular necrosis, hemorrhage and tubular degeneration (3b) and only mild hyperaemia (3c); necrosis; cellular infiltration, desquammation of mucosa (4b) and only mild infiltration of cells (4c).

alterations in group 3 animals which shows the non toxic nature of α-tocopherol at this dosage.

Levels of "TBA-reactants" in liver, kidney, heart and intestinal mucosa are given in Table I. There is a significant elevation in the levels of TBA reactants in all these organs in the order of Heart < intestine < liver < kidney. The formation of "TBA reactants" is significantly controlled in group 4 animals. Group 3 animals show significantly low levels of "TBA reactants" when compared to that of group 1 animals.

TABLE II: Activities of transaminases, lactate dehydrogenase and creatine kinase in the serum of control and experimental rats. Values are expressed as mean ± SD for 6 rats in each group.

Group	*Rats treated with*	*Glutamate oxaloacetate transaminase*	*Glutamate pyruvate transaminase*	*Lactate dehydrogenase*	*Creatine kinase*
1	None	27.3 ± 2.4	10.3 ± 2.7	82.6 ± 4.3	301.5 ± 5.2
2	Doxorubicin	37.4 ± 2.1**	14.4. ± 0.8**	103.0 ± 7.3*	362.0 ± 11.2**
3	α-tocopherol	25.1 ± 1.9^{NS}	11.2 ± 0.9^{NS}	77.5 ± 5.6^{NS}	312.2 ± 9.6^{NS}
4	Doxorubicin + α-tocopherol	29.3 ± 1.5^{NS}	9.25 ± 0.75^{NS}	90.0 ± 7.2^{NS}	290.0 ± 11.2*

The enzyme activities are expressed as I.U/litre, Statistically significant variations are derived by comparing group 2, 3 and 4 with group 1.

** P < 0.001 ** P < 0.01 NS - Non significant

Table II shows the activities of transaminases creatine kinase and lactate dehydrogenase in the serum of control and experimental animals. Activities of these enzymes are elevated in doxorubicin treated rats (Group 2). But the elevation is significantly minimum in group 4 rats. Group 3 rats didnot show any significant changes when compared to control rats.

Activities of intestinal Na^+, K^+ -dependent and Ca^{2+} -dependent ATPase and alkaline phosphatase are given in Table III Significant decreases are observed in the activities of these enzymes in doxorubicin treatment (Group 2). The alterations were minimum in α-tocopherol coadministered animals.

TABLE III Activitis of Na^+, K^+ ATPase, Ca^{++} ATPase and alkaline phosphatase in the intestinal mucosa of control and experimental rats. Values are expressed as mean ± SD for 6 rats in each group.

Group	*Rats treated with*	*Na^+, K^+ - ATP se activity*	*Ca^{++} -ATPase activity*	*Alkaline phosphatase activity*
1	None	4.6 ± 2.4	1.5 ± 0.10	3.8 ± 0.29
2	Doxorubicin	2.9 ± 0.21**	0.5. ± 0.02**	2.0 ± 0.21*
3	Vitamin-E	5.0 ± 0.52	1.7 ± 0.12	4.0 ± 0.32
4	Doxorubicin+ Vitamin-E	4.3 ± 0.41	1.2 ± 0.13*	3.3 ± 0.31*

The ATPase activities are expressed as micromoles of phosphorous liberated/min/mg protein. Alkaline phosphatase activity is expressed as micromoles of phenol liberated/min/mg protein. Statistically significant variations when compared to control are expressed as

**P < 0.001 *P <0.05 Non - significant

DISCUSSION

Doxorubicin has been shown to be a potential source of free radicals (16). Among the organs studied, heart is found to be more susceptible (Table I). Superoxide radicals and hydrogen peroxide radicals have been reported to be formed during quinone-semiquinone reactions involved

during doxorubicin metabolism (17). The necrotic changes observed here could have been attributed to the lipid peroxidative nature of doxorubicin.

Certain chemicals such as carbontetrachloride, chloroform and ethanol have been shown to induce necrosis in liver through lipid peroxidation (18,19). Intermediates such as conjugated dienes and hydroperoxides have been reported to be the primary causes.

Levels of transminases, creatine kinase and lactate dehydrogenase are the diagnostic indicators of hepatic and myocardial functions (20). Results from Table II clearly show the carditoxic and hepatotoxic nature of doxorubicin which have been demonstrated by various workers (2, 3).

As noted from the histological reports the hepatic necrosis and heart myocyte degeneration could have resulted in the leakage of enzymes into the blood stream. Generally, the quantity of enzyme released from the damaged tissue is a measure of number of necrotic cells. α-tocopherol co-administered rats show low levels of malonaldehyde in liver and heart and no necrosis. This is also reflected in the normal serum enzyme levels in group 4 animals. α-tocopherol is known to act as peroxyl radical-trapping chain breaking antioxidant and also act as a scavenger of free radicals (21, 22).

Nephrotoxicity has been reported in doxorubicin therapy (23). Histopathology of kidney from group 2 rats show glomerular necrosis and hemorrhage into the Bowman's capsule and tubular dengeration. α-tocopherol significantly minimize these changes.

The elevated lipid peroxidation in the intestinal mucosa would have been responsible for the observed decrease in the activities of membrane bound ATPases and alkaline phosphatase because biomembranes are more susceptible for free radical attack. Ca^{2+} and Na^{+}, K^{+} ATPases have been shown to be -SH group containing enzymes (24) and in many cases the inhibition of enzymes by quinone anticancer drugs was considered to be due to the attack on the -SH groups essential for the catalytic activity (25). Nicotera et al (26) have associated the loss of critical protein -SH groups with inactivation of Ca^{2+} dependent ATPase. It has been demostrated that the prevention of doxorubicin toxicity by α-tocopherol is due to maintenance of protein thiols (27). Alkaline phosphatase was reported to be a glycoprotein which were shown to be a preferential target for the active oxygen free radicals (28).

As is well known α-tocopherol is capable of protecting membrane lipids from lipid peroxidation *in vivo* (29) Diplock and Lucy (20) have demonstrated the interaction between phytyl side chain of α-tocopherol with membrane phosopholipid arachidonic acid which is the major site of lipid peroxidation.

From this investigation it could be supported that α-tocopherol prevents the peroxidative tissue damage induced by doxorubicin propably through its antioxidant capacity.

ACKNOWLEDGEMENTS

The authors (AG and RS) thank Council of Scientific and Industrial Research for the financial support. The authors are grateful to Dr. A. Sundara Rajan and Dr. A Thanigachalam, Department of Pathology, Veterinary College, Madras for the help rendered by them to the histopathology works.

REFERENCES

1. Blum RH, Carter SK. Adriamycin : A new anticancer drug with significant clinical activity. *Ann Int Med* 1974; 80: 249-59.

2. Van Hoff DD, Layard MW, Basa P, Von Hoff AL, Rosenweiz M, Muzio M. Risk factors for doxorubicin-induced congestive heart failure. *Ann Int Med* 1979; 91: 710-17.

3. Cortes EP, Lutman G, Wanka J, Wang JJ, Pickren J, Wallace J, Holland JF. Adriamycin (NSC 123127) Cardiotoxicity. A clinico Pathologic correlation. *Cancer Treat Rep* 1975; 6: 215-25.
4. Bachur NR, Gordon SL, Gee MV, Kon H. NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proc Natl Acad Sci* USA 1979; 76: 954-57.
5. Someya A, Tanaka N. DNA strand scission induced by adriamycin and Acacinomycin *Am J Antibiotics* 1979; 32: 839-45.
6. Myers CE, McGuire WP, Liss GH, Ifrim I, Grotzinger K, Young RC. Adriamycin : the role of lipid peroxidation in cardiotoxicity and tumor response. *Science* 1977; 19: 165-67.
7. Yamanaka N, Kato T, Nishida K, Fujikawa T, Fukushima M, Ota K. Elevation of Serum Lipid peroxide levels associated with doxorubicin toxicity and its amelioration by dl-α-tocopherol acetate or coenzyme Q_{10} in mouse. *Cancer chemother Pharmacol* 1979; 3: 223-27.
8. Richard JA. ADR Cardiotoxicity - contractile changes after long time treatment in the rats. *J. Pharmacol Exp. Ther* 1986; 236: 197-203.
9. King J. In "Practical clinical enzymology" 1965 D Von-Nostrand Co. Ltd, London.
10. Okinaka S, Kumagai H, Ebashi E, Sugaita M, Momoi Y, Tayokura Y, Fujie Y. Serum creatine phosphokinase-activity in progressive muscular dystrophy and neuromuscular diseases. *Arch Neurol* 1961; 4: 520.
11. Okhawa H, Ohishi N, Yagi K. Reaction of linoleic acid hydroperoxide with thiobarbituric acid. *Anal Biochem* 1979; 95: 351-54.
12. Fujita M, Matsui H, Nagato K, Nakao M. Asymmetric distribution of Quabain-sensitive activity in intestinal mucosa. *Biochem Biophys Acta* 1971; 233: 281-85.
13. Hjerton S, Pan H. Purification and Characterization of 2 forms of low affinity Ca^{++} ATPase from erythrocyte membrane. *Biochem Biophys Acta* 1983; 728: 281-88.
14. King EJ, Armstrong AR. In "Practical clinical chemistry" by Herold Varley 1976; 453-62.
15. Lowry OH, Rosebrough NJ, Farr LA, Randall RJ, A colorimetric method for protein determination. *J. Biol. Chem* 1951; 193: 265-75.
16. Doroshow JH. Effect of Anthracycline antibiotics on oxygen radical formation in heart. *Cancer Res* 1983; 43: 460-72.
17. Bachur NR, Gordon SL, Gee MV. A General mechanism of microsomal activation of quinone anticancer agents to free radicals. *Cancer Res* 1978; 38: 1745-50.
18. Slater TF, Sawyer BC. The stimulatory effects of CCl_4 and other halogeno alkanes on peroxidative reactions in rat liver fractions. *Biochem J* 1971; 123: 805-14.
19. Diluzio NR. The role of lipid peroxidation and antioxidants in ethanol induced lipid alterations. *Exp. mol Pathol* 1968; 8: 394-402.
20. Ewen LM, Griffiths J. 'Myocardial disorders'. *Am J Clin Pathol* 1971; 56: 614-18.
21. Tappel AL. Measurement of and protection from *in vivo* lipid peroxidation. In "Free radicals in Biology" by Pryor WA Academic Press, New York 1980; Vol 4: 1-47.
22. Machlin LJ. In "Vitamin E - a comprehensive treatise" Marcel Dekkar, New York, 1980.
23. Tullio B, Poggi A, Pozzoni R, Delaini F, Mecca G, Remuzzi G, Donati MB. ADR-induced nephrotic syndrome in rats - sequence of pathologic events. *Lab Invest* 1982; 46: 16-23.24.
24 Bellamo G, Mirabelli F, Richelmi P, Orrhenius S. Critical role of Sulphydryl in ATP dependent calcium sequestration by plasma membrane fraction from rat liver. *FEBS lett* 1983; 163: 136-39.
25. Ostenhof-Hoffman O. In "Metabolic Inhibitors-A comprehensive Treatise" (Eds - Hochester RM and Quastel JH). *Academic Press* 1963; Vol 11: p-145.
26. Nicotera P, Moore M, Mirabelli f, Bellamo G, Orrhensus S. Inhibition of hepatomite plasma membrane calcium ATPase activity in menadione metabolism and its restoration by thiols. *FEBS Lett.* 1985; 181: 149-52.
27. Pascoe GA, Reed RJ. Vitamin E protection against chemical-induced cell injury. *Arch Biochem Biophys* 1987; 256: 159-66.
28. Cooper B, Creeth JM, Donald ASR. Peroxidative damage in enzymes. *Biochem J* 1985; 228: 615-19.
29. Omar Hutsu H, Pratt CB. Vitamin E and membrane lipids. *Int J Radiat Oncol Biol Phys* 1979; 5: 1275-79.
30. Diplock AT, Lucy JA. The biochemical modes of action of vitamin E and selenium a hypothesis. *FEBS Lett* 1973; 20: 205-10

3. Cortes EP, Lutman G, Wanka J, Wang JJ, Pickren J, Wallace J, Holland JF. Adriamycin (NSC 123127) Cardiotoxicity. A clinico Pathologic correlation. *Cancer Treat Rep* 1975; 6: 215-25.
4. Bachur NR, Gordon SL, Gee MV, Kon H. NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proc Natl Acad Sci* USA 1979; 76: 954-57.
5. Someya A, Tanaka N. DNA strand scission induced by adriamycin and Acacinomycin *Am J Antibiotics* 1979; 32: 839-45.
6. Myers CE, McGuire WP, Liss GH, Ifrim I, Grotzinger K, Young RC. Adriamycin : the role of lipid peroxidation in cardiotoxicity and tumor response. *Science* 1977; 19: 165-67.
7. Yamanaka N, Kato T, Nishida K, Fujikawa T, Fukushima M, Ota K. Elevation of Serum Lipid peroxide levels associated with doxorubicin toxicity and its amelioration by dl-α-tocopherol acetate or coenzyme Q_{10} in mouse. *Cancer chemother Pharmacol* 1979; 3: 223-27.
8. Richard JA. ADR Cardiotoxicity - contractile changes after long time treatment in the rats. *J. Pharmacol Exp. Ther* 1986; 236: 197-203.
9. King J. In "Practical clinical enzymology" 1965 D Von-Nostrand Co. Ltd, London.
10. Okinaka S, Kumagai H, Ebashi E, Sugaita M, Momoi Y, Tayokura Y, Fujie Y. Serum creatine phosphokinase-activity in progressive muscular dystrophy and neuromuscular diseases. *Arch Neurol* 1961; 4: 520.
11. Okhawa H, Ohishi N, Yagi K. Reaction of linoleic acid hydroperoxide with thiobarbituric acid. *Anal Biochem* 1979; 95: 351-54.
12. Fujita M, Matsui H, Nagato K, Nakao M. Asymmetric distribution of Quabain-sensitive activity in intestinal mucosa. *Biochem Biophys Acta* 1971; 233: 281-85.
13. Hjerton S, Pan H. Purification and Characterization of 2 forms of low affinity Ca^{++} ATPase from erythrocyte membrane. *Biochem Biophys Acta* 1983; 728: 281-88.
14. King EJ, Armstrong AR. In "Practical clinical chemistry" by Herold Varley 1976; 453-62.
15. Lowry OH, Rosebrough NJ, Farr LA, Randall RJ, A colorimetric method for protein determination. *J. Biol. Chem* 1951; 193: 265-75.
16. Doroshow JH. Effect of Anthracycline antibiotics on oxygen radical formation in heart. *Cancer Res* 1983; 43: 460-72.
17. Bachur NR, Gordon SL, Gee MV. A General mechanism of microsomal activation of quinone anticancer agents to free radicals. *Cancer Res* 1978; 38: 1745-50.
18. Slater TF, Sawyer BC. The stimulatory effects of CCl_4 and other halogeno alkanes on peroxidative reactions in rat liver fractions. *Biochem J* 1971; 123: 805-14.
19. Diluzio NR. The role of lipid peroxidation and antioxidants in ethanol induced lipid alterations. *Exp. mol Pathol* 1968; 8: 394-402.
20. Ewen LM, Griffiths J. 'Myocardial disorders'. *Am J Clin Pathol* 1971; 56: 614-18.
21. Tappel AL. Measurement of and protection from *in vivo* lipid peroxidation. In "Free radicals in Biology" by Pryor WA Academic Press, New York 1980; Vol 4: 1-47.
22. Machlin LJ. In "Vitamin E - a comprehensive treatise" Marcel Dekkar, New York, 1980.
23. Tullio B, Poggi A, Pozzoni R, Delaini F, Mecca G, Remuzzi G, Donati MB. ADR-induced nephrotic syndrome in rats - sequence of pathologic events. *Lab Invest* 1982; 46: 16-23.24.
24 Bellamo G, Mirabelli F, Richelmi P, Orrhenius S. Critical role of Sulphydryl in ATP dependent calcium sequestration by plasma membrane fraction from rat liver. *FEBS lett* 1983; 163: 136-39.
25. Ostenhof-Hoffman O. In "Metabolic Inhibitors-A comprehensive Treatise" (Eds - Hochester RM and Quastel JH). *Academic Press* 1963; Vol 11: p-145.
26. Nicotera P, Moore M, Mirabelli f, Bellamo G, Orrhensus S. Inhibition of hepatomite plasma membrane calcium ATPase activity in menadione metabolism and its restoration by thiols. *FEBS Lett.* 1985; 181: 149-52.
27. Pascoe GA, Reed RJ. Vitamin E protection against chemical-induced cell injury. *Arch Biochem Biophys* 1987; 256: 159-66.
28. Cooper B, Creeth JM, Donald ASR. Peroxidative damage in enzymes. *Biochem J* 1985; 228: 615-19.
29. Omar Hutsu H, Pratt CB. Vitamin E and membrane lipids. *Int J Radiat Oncol Biol Phys* 1979; 5: 1275-79.
30. Diplock AT, Lucy JA. The biochemical modes of action of vitamin E and selenium a hypothesis. *FEBS Lett* 1973; 20: 205-10