2. ACUTE STUDIES

2.1 INTRODUCTION

Cancer chemotherapeutic agents characteristically have a narrow therapeutic indice with toxic side effects. A toxic effect is one that occurs as an exaggeration of the desired therapeutic effect and which is not common at normal doses (Edwards and Aronson, 2000). A vast number of circumstantial evidence implicates oxygen derived radicals as mediators of toxic side effects of antineoplastic drugs.

ADR is an anthracycline antineoplastic agent used in the treatment of a variety of human neoplasms (Saad et al., 2001). Despite its dose limiting side effects it continues to be one of the most widely used anticancer agents in the clinic. Many of ADR's toxicities occur due to its generation of toxic oxygen species, resulting in oxidative stress (Pritsos and Ma, 2000). Though oxidative damage in diseased tissues such as tumor can lead to its cure, its selective inducement in normal tissues can lead to health hazards.

Therapeutic efficacy of ADR in the treatment of human cancer is limited by both its acute and chronic side effects. Excessive generation of ROS contributes to the process of progressive renal injury in a variety of clinical and experimental renal diseases (Ichikawa et al., 1994). Nephrotoxicity is a serious limiting factor for clinical application of ADR in cancer chemotherapy.

Biological reduction of the drug produces a semiquinone metabolite which subsequently reacts with molecular oxygen to form ROS (Bachur et al., 1977).
These oxygen metabolites play a significant role in altering glomerular ultrafiltration through membrane peroxidation with subsequent membrane destabilization and leakage (Baud et al., 1981). Under anoxic conditions, the ADR semiquinone radical can rearrange to a species which can alkylate both proteins (Ghezzi et al., 1968) and nucleic acids (Sinha and Gregory, 1981).

In view of the generation of ROS practically all the crucial biomolecules undergo an oxidative reaction leading to biochemical disturbances (Mimnaugh et al., 1985). Partially reduced ROS are extremely cytotoxic since they can react with and damage enzymes (Freeman and Crapo, 1982), nucleic acids (Brawn and Fridovich, 1981) and lipids (Bus and Gibson, 1979). ROS are kept in check by various levels of antioxidants. However enhancement in the generation of ROS can deplete the antioxidant defences (Figure 2.1).

Nephrotoxicity of aminoglycosides appears to be closely related to the intracellular accumulation of the drug within the renal cortex. Following infiltration by the glomerulus, a small amount of the filtered aminoglycoside is reabsorbed from the proximal tubular cells and within lysosomes in the tubular cells. Cortical aminoglycoside uptake and nephron damage is directly correlated with serum aminoglycoside concentration (Chan, 1989). On balance, the available data suggests that nephrotoxicity is directly correlated with a combination of duration and daily dose.

Successful treatment of renal disease remains a frustrating aspect of clinical nephrology. Biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effect of ROS and other radicals (Ciaccio et al., 1993). LA is one such well known antioxidant. Despite the
Figure 2.1 Schematic representation which shows the activation of adriamycin by mitochondrial NADH-dehydrogenase to establish the drug semiquinone-quinone redox cycle in the presence of molecular oxygen. Also shown is the reductive activation of oxygen to the superoxide anion free radical and the cascade of reactions and interactions, which generate secondary reactive oxygen species, which eventually results in greatly enhanced peroxidation of unsaturated mitochondrial membrane phospholipids (Mimnaugh et al., 1985).
wide therapeutic use of LA in many pathologies, little is known about its effectiveness in thwarting the ADR induced toxicity. In the present study, an attempt was therefore made to investigate in detail the adverse effects on various biochemical parameters associated with ADR induced nephrotoxicity in rat model and the effect of lipoic acid in their amelioration.

2.2 MATERIALS AND METHODS

2.2.1 Materials

DL α-lipoic acid was procured from Sigma Chemicals, St. Louis, USA. All other chemicals were of analytical grade procured from local commercial sources.

2.2.2 Animal model

Adult male Wistar rats weighing 150 ± 20g (12-14 week old) obtained from Tamilnadu University for Veterinary and Animal Sciences, Chennai, India, were used throughout the experiment. The animals were housed under conditions of controlled temperature (26 ± 2°C) with 12-hour day-night cycle. They were fed standard rat chow (M/s.Pranav Agro Industries Ltd., India) and were given access to water ad libitum.
2.2.3 **Sources of fine chemicals**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dithio nitrobenzoic acid</td>
<td>BDH Chemicals Limited, Poole, England</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Sisco Research Laboratories Private Limited, Bombay, India.</td>
</tr>
<tr>
<td>2-Oxoglutarate (sodium salt)</td>
<td></td>
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<tr>
<td>Adenosine triphosphate</td>
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<tr>
<td>Nicotinamide adenine dinucleotide</td>
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<tr>
<td>Tris</td>
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<tr>
<td>Galactosamine hydrochloride</td>
<td></td>
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<tr>
<td>Glucose-6-phosphate</td>
<td></td>
</tr>
<tr>
<td>DL α-Lipoic acid</td>
<td>Sigma Chemicals, St.Louis, USA.</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
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</tr>
<tr>
<td>L-γ-Glutamyl-p-nitroanilide</td>
<td></td>
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<tr>
<td>Alcian blue 8GX</td>
<td></td>
</tr>
<tr>
<td>Dialysis sacs</td>
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<tr>
<td>L-Leucine-β-napthylamide</td>
<td>Dabur India Ltd., New Delhi, India.</td>
</tr>
<tr>
<td>N-Acetyl-neuraminic acid</td>
<td>Loba-Chemie, Mumbai, India.</td>
</tr>
<tr>
<td>Doxorubicin hydrochloride (ADRIM)</td>
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</tr>
<tr>
<td>Lithium lactate</td>
<td>Fluka, A.G., Buchs, S.G., Switzerland.</td>
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<tr>
<td>Orcinol</td>
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<tr>
<td>Silicic acid</td>
<td>Sarabhai M. Chemicals, Baroda</td>
</tr>
<tr>
<td>Sodium periodate</td>
<td>L.H.Boehringer, Ingelhelm, Germany</td>
</tr>
<tr>
<td>Tripalmitin</td>
<td>E.Merck, Darmstadt, Germany.</td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>Koch-Light Laboratories, Coln Brook, England</td>
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<tr>
<td>Fructose-1,6-diphosphate</td>
<td>S.D. Fine Chemicals, Mumbai, India.</td>
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<tr>
<td>2-4-Aminonaphthol sulphonic acid</td>
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<tr>
<td>Sodium pyruvate</td>
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<tr>
<td>Chromotropic acid</td>
<td></td>
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<tr>
<td>Sodium meta arsenite</td>
<td></td>
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<tr>
<td>1-Chloro-2,4-dinitrobenzene</td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td></td>
</tr>
<tr>
<td>Diphenyl amine</td>
<td></td>
</tr>
<tr>
<td>Bathophenanthroline</td>
<td></td>
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<tr>
<td>Thiobarbituric acid</td>
<td></td>
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</tbody>
</table>

All other chemicals including solvents used were also of highest purity and analytical grade.
2.2.4 Experimental design

The animals were divided into four groups of six rats each as follows:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal rats, injected with 0.5 ml physiological saline, formed the control group.</td>
</tr>
<tr>
<td>II</td>
<td>Administered a single dose of adriamycin (7.5 mg per kg body weight i.v.) through tail vein.</td>
</tr>
<tr>
<td>III</td>
<td>Administered a single dose of lipoic acid (75 mg per kg body weight i.p.).</td>
</tr>
<tr>
<td>IV</td>
<td>Administered lipoic acid 24 hours prior to the administration of adriamycin.</td>
</tr>
</tbody>
</table>

2.2.5 Collection of rat urine

The rats were housed in metabolic cages for 24 hr urine collection. The rats were provided with water but no feed was given. A 50 ml beaker, maintained at 0°C in an ice bath, was used for collection. The urine collected was free from faecal contamination.

a) A portion of the sample was acidified with concentrated HCl and used for the analysis of urea, uric acid, creatinine and phosphorous after centrifuging for 10 min to remove sediments.

b) A known aliquot was set aside for the estimation of total GAGs as alcian blue precipitable polyanions.

c) The remaining portion was dialysed at 4°C against distilled water for 3 hrs. Aliquots of the dialysed urine were then used for the determination of enzymes and protein.
2.2.6 Collection of rat tissues

At the end of the experimental period, the animals were killed by cervical decapitation under ether anaesthesia. Kidneys were excised immediately and washed with ice-cold saline and their weights recorded.

2.2.7 Preparation of tissue homogenate

A 10% homogenate of the washed tissue (kidneys) was prepared in 0.01M phosphate buffer, pH 7.0, for all the enzymes, except for ATPases, for which 0.1M Tris-HCl buffer of pH 7.4 was used.

2.2.8 Assay of tissue enzymes
2.2.8.1 Alkaline phosphatase (EC 3.1.3.1, ALP) and Acid phosphatase (EC 3.1.3.2, ACP)

The procedure of King (1965a) was used to estimate the enzyme activity using disodium phenyl phosphate as substrate. The colour developed was measured at 640 nm. The activities of ALP and ACP was expressed as μ moles of phenol liberated per min per mg protein.

2.2.8.2 Lactate dehydrogenase (EC 1.1.1.27, LDH)

The method adopted for the estimation of LDH was that of King (1965b). The method was based on the ability of LDH to convert lactate to pyruvate with the help of the co-enzyme NAD⁺. The pyruvate formed was made to react with 2,4-dinitrophenyl hydrazine in hydrochloric acid. The hydrazone formed turns into an orange coloured complex in alkaline medium which was measured at 420 nm.
Enzyme activity was expressed as μ moles of pyruvate formed per min per mg protein.

2.2.8.3 Cathepsin-D (EC 3.4.23.5, Cat-D)

Cat-D was determined by the method of Biber et al. (1981). Enzyme activity was expressed as n moles of tyrosine released per mg protein per hr.

2.2.8.4 N-acetyl-β-D-glucosaminidase (EC 3.2.1.30, NAG)

The method of Maruhn (1976) was followed for the determination of NAG activity. The colour developed was read at 420 nm. The enzyme activity was expressed as units per mg creatinine; a unit is defined as n moles of p-nitrophenol released per hr.

2.2.8.5 β-Glucuronidase (β-D-glucuronide glucuronosidase hydrolase, EC 3.2.1.31, β-Glu)

β-Glucuronidase was estimated by the method of Kawai and Anno (1971). The colour developed was read at 400 nm. The activity was expressed as μ moles of p-nitrophenol liberated per mg creatinine per hr.

2.2.8.6 β-Galactosidase (EC 3.2.1.23, β-Gal)

β-Galactosidase was estimated by the method of Rosenblit et al. (1974). The enzyme activity was expressed as units per mg protein, a unit is defined as μ moles of p-nitrophenol formed per mg protein per hour.
2.2.8.7 5'-Nucleotidase (5-ribonucleotide phosphohydrolase, EC 3.1.3.5, 5'-AMP)

5'-Nucleotidase was assayed by the method of Luly et al. (1972). The enzyme activity was expressed as μ moles of phosphorus liberated per mg protein per min.

2.2.8.8 Protein content

Protein content was estimated by the method of Lowry et al., 1951. The blue colour formed was measured at 640 nm after 15 min against blank. Protein values was expressed as mg per g wet tissue; Protein values in urine was expressed as mg per 24 hrs.

2.2.8.9 Phosphorus

The phosphorus content was estimated by the method of Fiske and Subbarow (1925).

2.2.9 Amino transferases

2.2.9.1 Aspartate transaminase (EC 2.6.1.1, AST)

The enzyme catalyses the reaction:

L-aspartate + 2-oxoglutarate ---→ oxaloacetate + L-glutamate

It was assayed by the method of King (1965c). The colour developed was read at 540 nm. The enzyme activity was expressed as μ moles of pyruvate liberated per min per mg protein.
2.2.9.2 Alanine transaminase (EC 2.6.1.2, ALT)

The enzyme catalyses the reaction:
L-alanine + 2-oxoglutarate \(\rightarrow\) pyruvate + L-glutamate

This enzyme was estimated by the method of King (1965c). The colour developed was read at 540 nm. The enzyme activity was expressed as \(\mu\) moles of pyruvate liberated per min per mg protein.

2.2.10 Nucleic acids

Nucleic acids were extracted from tissues by the method of Schneider (1945).

2.2.10.1 Deoxyribonucleic acid (DNA)

DNA was estimated according to the method of Burton (1956) with diphenylamine. Blue colour developed was measured at 640 nm. DNA content was expressed as mg per g wet tissue.

2.2.10.2 Ribonucleic acid (RNA)

RNA was estimated by the method of Rawal et al. (1978). The colour developed was read at 640 nm. The value was expressed as mg per g wet tissue.
2.2.11 Glycolytic enzymes

2.2.11.1 Hexokinase (ATP : D-hexose-6-phosphotransferase, EC 2.7.1.1, HK)

This enzyme was assayed by the method of Branstrup et al. (1957) by determining the rate of disappearance of glucose at 37°C. The enzyme activity was expressed as n moles of glucose utilised for the formation of glucose-6-phosphate per min per mg protein.

2.2.11.2 Phosphoglucoisomerase (D-glucose-6-phosphate: Ketol isomerase, EC 5.3.1.9, PGI)

The enzyme was assayed by the method of Horrocks et al. (1963). The assay is based on the estimation of fructose by the resorcinol-thiourea reagent. The colour developed was immediately read at 410 nm in a Klett-Summerson photoelectric colorimeter. Specific activity is expressed as n moles of fructose formed per min per mg protein.

2.2.11.3 Aldolase (D-Fructose-1,6 bis phosphate D-glyceraldehyde-3-phosphate lyase, EC 4.1.2.1.3)

This enzyme was assayed by the method of King (1965c). The enzyme activity was expressed as n moles of glyceraldehyde formed per min per mg protein.
2.2.12 Gluconeogenic enzymes

2.2.12.1 Glucose-6-phosphatase (Glucose-6-phosphatase phosphohydrolase, EC 3.1.3.9, G6P)

Glucose-6-phosphatase was assayed according to the method of King (1965a). This enzyme catalyses the reaction:

\[
\text{Glucose-6-phosphate} + H_2O \rightarrow \text{Glucose} + \text{Pi}
\]

The phosphorous content was estimated as in section 2.2.8.9. The enzyme activity was expressed as n moles of Pi liberated per min per mg protein.

2.2.12.2 Fructose-1,6-diphosphatase (Fructose-1,6-diphosphate phosphohydrolase, EC 3.1.3.11, FDP)

Fructose-1,6-diphosphatase was assayed by an adaptation of the procedure described by Gancedo and Gancedo (1971).

This enzyme catalyses the reaction:

\[
\text{Fructose-1,6-phosphate} + H_2O \rightarrow \text{Fructose 6-phosphate} + \text{Pi}
\]

The phosphorus content was estimated as in section 2.2.8.9. The enzyme activity was expressed as n moles of Pi liberated per min per mg protein.
2.2.13 Glycogen and glycogen phosphorylase

2.2.13.1 Glycogen

Glycogen was extracted and estimated according to the method of Morales et al. (1973). The glycogen content in the tissues was expressed as mg per g tissue.

2.2.13.2 Glycogen phosphorylase (EC 2.4.1.1)

Glycogen phosphorylase was assayed by the method of Hers and Van Hoof (1966). The phosphorous content was estimated as in section 2.2.8.9. Enzyme unit was expressed as μ moles of Pi liberated per min per mg protein.

2.2.14 Phosphohydrolases

2.2.14.1 Total adenosine triphosphatase (ATP-phosphohydrolase, EC 3.6.1.3, Total ATPase)

The activity of the enzyme was measured by the method of Evans (1970).

2.2.14.2 Na⁺, K⁺-adenosine triphosphatase (ATP-phosphohydrolase, EC 3.6.1.3)

Na⁺,K⁺-ATPase activity was estimated by the method of Bonting (1970). The phosphorus was estimated as in section 2.2.8.9. The enzyme activity was expressed as μ moles of Pi liberated per min per mg protein.
2.2.14.3 Mg\(^{2+}\) - adenosine triphosphatase (EC 3.6.1.3)

The activity of the enzyme was estimated according to the method of Ohnishi et al. (1982). The phosphorous content was estimated as in section 2.2.8.9. The enzyme activity was expressed as \(\mu\) moles of Pi liberated per min per mg protein.

2.2.14.4 Ca\(^{2+}\)-adenosine triphosphatase (EC 3.6.1.3)

The activity of the enzyme was estimated according to the method of Hjerten and Pan (1983). Phosphorous content was estimated as in section 2.2.8.9. The enzyme activity was expressed as \(\mu\) moles of Pi liberated per min per mg protein.

2.2.15 TCA cycle enzymes

2.2.15.1 Succinate dehydrogenase (Succinate: (acceptor) Oxido reductase, EC 1.3.99.1, SDH)

This method is a modification of the method of Slater and Bonner (1952). The change in OD at 340 nm was measured for 2 min at intervals of 15 sec. Enzyme activity was expressed as \(\mu\) mole of succinate produced per min per mg protein.

2.2.15.2 Malate dehydrogenase (L-Malate : NAD : Oxido reductase, EC 1.1.1.37, MDH)

The method adopted for the estimation of MDH was that of Mehler et al. (1948). The activity of the enzyme was expressed as n moles of NADH oxidised per
min per mg protein using the extinction co-efficient of NADH as $6.22 \times 10^{-6} \text{ cm}^2 \text{ mole}^{-1}$.

2.2.15.3 Isocitrate dehydrogenase (threo-D$_3$-Isocitrate NADP$^+$ Oxido Reductase (decarboxylating), EC 1.1.1.42, ICDH)

The method adopted for the estimation of ICDH was that of King (1965b). The colour intensity was read at 420 nm for 10 min in a spectrophotometer. The activity of the enzyme was expressed as nanomoles of $\alpha$-ketoglutarate produced per min per mg protein.

2.2.16 Lipid peroxidation (LPO)

Tissue LPO was measured by the method of Devasagayam (1986). The malondialdehyde content of the samples was expressed as n moles of MDA formed per mg protein.

2.2.16.1 Ascorbate-induced lipid peroxidation

Ascorbate-induced LPO was carried out as in section 2.2.16. The peroxidation system contained 0.2 ml of a solution containing ferric chloride (3.4 mg), ADP (9.8 mg) and ascorbate (8.8 mg) in 2 ml.

2.2.16.2 Ferrous sulphate-induced lipid peroxidation

Ferrous sulphate-induced LPO was also carried out in section 2.2.16. The peroxidation system contained 0.2 ml of 10 mM ferrous sulphate.
2.2.17 Tissue antioxidants

2.2.17.1 Total reduced glutathione (GSH)

Total reduced glutathione was determined by the method of Moron et al. (1979). This method was based on the reaction of reduced glutathione with dithiobis nitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. The amount of glutathione was expressed as μg per mg protein.

2.2.17.2 Vitamin E (Vit E)

Vitamin E was estimated by the method of Desai (1984). The absorbance was read at 536 nm. Vitamin E values were expressed as mg per g tissue.

2.2.17.3 Ascorbic acid (Vit C)

Ascorbic acid was estimated by the method of Omaye et al. (1971). The absorbance was read at 520 nm. Ascorbic acid values were expressed as μg per mg protein.

2.2.17.4 Vitamin A (Vit A)

Vitamin A was estimated by the method of Bayfield and Cole (1974). Vitamin A level was expressed as mg per g tissue.

2.2.18 Tissue antioxidizing enzymes

2.2.18.1 Catalase (EC 1.11.1.6; CAT)

The activity of catalase was assayed by the method of Sinha (1972). Catalase causes rapid decomposition of hydrogen peroxide to water.
Principle

Dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H₂O₂) with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus formed was measured at 610 nm. The activity of catalase was expressed as μ moles of H₂O₂ consumed per min per mg protein.

2.2.18.2 Superoxide dismutase (EC 1.15.1.1, SOD)

The enzyme was assayed according to the method of Marklund and Marklund (1974).

Principle

The degree of inhibition of auto-oxidation of pyrogallol, at an alkaline pH, by superoxide dismutase was used as a measure of the enzyme activity. The enzyme activity was expressed as units per mg protein where one unit of enzyme activity was defined as the amount of enzyme required to give 50% inhibition of pyrogallol auto-oxidation.

2.2.18.3 Glutathione peroxidase (EC 1.11.1.9, GPx)

The activity of glutathione peroxidase (GPx) was determined by the method of Rotruck et al. (1973) with some modifications. The colour developed was read at 412 nm. The enzyme activity was expressed as μg of GSH consumed per min per mg protein.
2.2.18.4 Glutathione reductase (EC 1.6.4.2, GR)

The enzyme was assayed by the method of Beutler (1984).

\[
\text{NADPH (NADH) + H}^+ + \text{GSSG} \rightarrow \text{NADP}^+ (\text{NAD})^+ + 2\text{GSH}
\]

The decrease in OD at 340 nm, at 37°C of the system was measured against the blank in a Uvikon 90 spectro photometer for 3 min at 1 min intervals. Activity of GR was expressed as units per mg protein.

2.2.18.5 Glutathione-S-transferase (EC 2.5.1.18, GST)

This enzyme was assayed by the method of Habig et al. (1973). The increase in OD if the enzyme was measured against that of the blank at 340 nm. Enzyme activity was expressed as nanomoles of CDNB-GSH conjugate formed per min per mg protein.

2.2.18.6 Glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PD)

This enzyme was assayed by the method of Balansky and Bernstein (1963). Though the activity of this enzyme is not dependent on glutathione, it produces reducing equivalents which are essential for the activity of reductases. Increase in OD was measured at 340 nm. The activity of the enzyme was expressed as nanomoles of NADP (H) formed per min per mg protein.

2.2.18.7 Xanthine oxidase (EC 1.2.3.2, XO)

Xanthine oxidase activity was determined by the method of Fried and Fried (1966). The increase in the intensity of colour with time was determined at
32 nm as a measure of the enzyme activity. The activity of the enzyme is expressed in terms of units per mg protein in which one unit was equal to the amount of enzyme that brought about a change in OD of 0.01 per min.

### 2.2.19 Glutathione metabolising enzymes

#### 2.2.19.1 γ-glutamyl transpeptidase (EC 2.3.2.2, γ-GT)

This enzyme was estimated by the method of Orlowski and Meister (1965). This enzyme catalyses the transfer for γ-glutamyl groups from γ-glutamyl donors to amino acids, peptide or water. One unit of enzyme activity was defined as μ moles of p-nitroaniline released per min per mg protein.

#### 2.2.19.2 γ-glutamyl cysteine synthetase (EC 6.3.2.2, γ-GCS)

γ-glutamyl cysteine synthetase activity was determined by the method of Mooz and Meister (1971).

**Principle**

The amount of inorganic phosphorus released by the action of this enzyme is estimated colorimetrically.

\[
\text{γ-GCS} \quad \text{L-glutamic acid+L-cysteine+ATP} \quad \rightarrow \quad \text{L-γ-glutamyl-L-cysteine+ADP+Pi}
\]

The activity of the enzyme was expressed as μg of Pi liberated per min per mg protein.
2.2.20 Protein bound carbohydrates

A known amount of defatted residues of liver and kidney were hydrolysed with 2 ml of 3N HCl at 80°C for 4 hr. The hydrolysed material was neutralised and used for the estimation of hexose, hexosamine and hexuronic acid.

2.2.20.1 Hexose

Hexose was estimated by the method of Niebes (1972). The absorbance was read at 540 nm against a reagent blank. The hexose content was expressed as mg per 100 mg defatted tissue.

2.2.20.2 Hexosamine

Hexosamine was estimated by the method of Wagner (1979). The pink colour developed was measured at 540 nm against the blank. Hexosamine content of tissue was expressed as mg per 100 mg defatted tissue.

2.2.20.3 Hexuronic acid

Hexuronic acid was estimated by the method of Bitter and Muir (1962). The colour developed was read at 530 nm. The values were expressed as mg per 100 mg defatted tissue.

2.2.20.4 Sialic acid

Sialic acid was determined by the method of Aminoff (1961) with modification by Niebes (1972). A known amount (50 mg) of defatted residue of liver and kidney was hydrolysed with 1 ml of 0.1N H$_2$SO$_4$ at 80°C for 60 min. The
hydrolysed material was neutralised and used for the estimation of sialic acid. The values were expressed as μg per 100 mg defatted tissue.

2.2.21 Tissue lipids

2.2.21.1 Extraction of lipids

Lipids were extracted from the liver and kidney according to the method of Folch et al. (1957). Total lipids were expressed as mg per g wet tissue.

2.2.21.2 Cholesterol (TC)

Cholesterol was estimated by the method of Jung (1975). The colour developed was read at 540 nm after 15 min. Cholesterol content was expressed as mg per g wet tissue.

2.2.21.3 Free cholesterol (FC)

The cholesterol was precipitated as its digitonide according to the method of Sperry and Webb (1950) and cholesterol in the precipitate was estimated by the method of Jung (1975). The colour developed was read at 540 nm after 20 min. The ester cholesterol was arrived at from the difference between the total and free cholesterol analysed. Values of free and esterified cholesterol were expressed as mg per g wet tissue.
2.2.21.4 Triglycerides (TG)

Triglycerides were estimated by the method of Rice (1970). The colour developed was read at 570 nm against the reagent blank. Triglycerides were expressed as mg per g wet tissue.

2.2.21.5 Phospholipids (PL)

Phospholipids was estimated by the method of Rouser et al. (1970) after digesting the lipid extract with perchloric acid (Barlett, 1959). The colour developed was read immediately at 700 nm. Phosphorus content was multiplied by a factor 25 which gave the weight of phospholipids. Phospholipids were expressed as mg per g wet tissue.

2.2.21.6 Free fatty acids (FFA)

Free fatty acids was measured by the method of Horn and Menahan (1981). The colour developed was read immediately at 430 nm against a reagent blank. Free fatty acid content were expressed as mg per g wet tissue.

2.2.22 Cholesterol metabolising enzymes

2.2.22.1 Cholesterol ester hydrolase (EC 3.1.1.13, CEH)

Cholesterol ester hydrolase activity was estimated by the method of Kothari et al. (1970) with slight modification of Kritchevsky and Kothari (1973). The enzyme activity was expressed as n moles of cholesterol liberated per mg protein per hr.
2.2.22.2 Cholesterol ester synthetase (EC 2.3.1.26, CES)

This enzyme was analysed by the method of Kothari et al. (1973). Activity was expressed as n moles of cholesterol esterified per mg protein per hr.

2.2.22.3 Lipoprotein lipase (EC 3.1.1.3, LPL)

The lipoprotein lipase was assayed by the method of Schmidt (1974) and was expressed as µ moles of free fatty acids liberated per mg protein per hr.

2.2.23 Estimation of serum lipids and lipoproteins

2.2.23.1 Serum cholesterol, free cholesterol, triglycerides, phospholipids and free fatty acids were estimated as in section 2.2.21.2, 2.2.21.3, 2.2.21.4, 2.2.21.5, 2.2.21.6 respectively.

2.2.23.2 Lipoproteins

Reagents

1. Heparin-manganese chloride reagent: 3.167 g of MnCl₂ was added to 1 ml solution of heparin containing 20,000 units. This mixture was made up to 8 ml with distilled water.

2. Sodium dodecyl sulphate: 10% in 0.15M sodium chloride.

Procedure

Lipoproteins were fractionated by a dual precipitation technique (Wilson and Spiger, 1973). Addition of heparin-MnCl₂ to serum caused precipitation of
VLDL and LDL. The supernatant obtained after sedimentation represented the HDL fraction.

1 ml of serum was added to 0.18 ml of heparin-MnCl₂ reagent and mixed well. The solution was allowed to stand at 4°C for 30 min and then centrifuged in a refrigerated centrifuge at 2,500 x g maintained at 10°C for 30 min. The supernatant containing the HDL fraction was used for estimation of cholesterol by the method of Jung (1975) as in section 2.2.21.2.

To an aliquot of serum, sodium dodecyl sulphate (SDS) was added which resulted in the aggregation of VLDL which flocculated on top. The supernatant contained both HDL and LDL (Burstein and Scholnick, 1972). 1 ml of serum was added to 0.15 ml of sodium dodecyl sulphate. The contents were centrifuged in a refrigerated centrifuge at 10,000 x g for 15 min and the cholesterol content in the supernatant was estimated.

An aliquot of serum was assayed for total cholesterol.

The cholesterol content of each fraction was arrived in the following manner:

\[
\text{Total cholesterol} - \text{supernatant from SDS} = \text{VLDL cholesterol containing HDL and LDL}
\]
\[
\text{Supernatant from SDS - heparin Mn}^{2+} \text{supernatant} = \text{LDL cholesterol (HDL)}
\]

Lipoprotein cholesterol was expressed as mg per dl of serum.
2.2.24 Biochemical investigations in urine

2.2.24.1 Urea

Urea was estimated by the method of Natelson et al. (1951). The reaction mixture was read at 480 nm. The values were expressed as mg per 24 hr.

2.2.24.2 Uric acid

Uric acid was estimated by the method of Caraway (1963). The colour was read at 640 nm after 10 min. The values were expressed as mg per 24 hr.

2.2.24.3 Creatinine

Creatinine content was estimated by the method of Owen et al. (1954). The colour developed was read immediately at 540 nm after 15 min. The results were expressed as mg of creatinine per dl.

2.2.24.4 Protein

The protein content was determined as in section 2.2.8.8.

2.2.24.5 Inorganic phosphorus

Inorganic phosphorous was estimated as in section 2.2.8.9. The values were expressed as mg of Pi per 24 hr.

2.2.24.6 Total glycosaminoglycans (GAGs)

Total GAGs in urine was determined by the method of Whiteman (1973) with slight modifications (Hwang et al., 1988). The extinction of the clear blue
solution was read at 620 nm in 1 cm micro cuvettes in a spectrophotometer. This method is based on the interaction of cationic dye alcian blue 8GX with acidic glycosaminoglycans. Results were expressed as mg of GAGs excreted per 24 hr.

2.2.25 Biochemical estimations in serum

Serum urea, uric acid and creatinine was estimated according to the procedure in sections 2.2.24.1, 2.2.24.2, 2.2.24.3 respectively.

2.2.26 Assay of urinary enzymes

2.2.26.1 Urinary enzymes ALP, ACP, LDH, NAG, β-glu, β-Gal, γ-GT were estimated as in sections 2.2.8.1, 2.2.8.1, 2.2.8.2, 2.2.8.4, 2.2.8.5 and 2.2.8.6 and 2.2.19.1 respectively.

2.2.26.2 Leucine aminopeptidase (L-leucyl-peptide hydrolase, EC 3.4.1.2, LAP)

The leucine aminopeptidase (LAP) activity was measured by the method of Natelson (1971) using L-leucyl-β-naphthylamide as the substrate. The purple colour formed was read after 25 min at 560 nm. The enzyme activity was expressed as μ moles of β-naphthylamide formed per mg creatinine per hr.

2.2.7 Histopathological studies

A portion of the tissue (kidney), immediately after sacrifice was kept in 10% formalin for at least 24 hr to fix the tissue. The tissues were washed in running tap water, dehydrated in the descending grades of isopropanol and finally cleared in xylene. The tissues were then embedded in molten paraffin wax.
Sections were cut at 7 μm thickness, stained with haematoxylin and eosin. The sections were then viewed under light microscope for histopathological changes in the kidney.

2.2.8 Statistical analysis

The values are expressed as mean ± standard deviation. The results were computed statistically (SPSS Software package) using one-way analysis of variance. Post hoc testing were performed for inter-group comparisons using the Least Significance Difference (LSD) test; A p value < 0.05 was considered significant.

2.3 RESULTS AND DISCUSSION

2.3.1 Tissue enzymes

The effect of ADR and LA administrations on tissue enzymes is depicted in table 2.1. Significant alterations were observed in the activity of all the enzymes studied except ACP.

ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. There are two distinct forms of ALP, a bone/liver/kidney/placental form and an intestinal form. ALP has a broad substrate specificity and catalyses the hydrolysis of a number of phosphate esters presumably via a phosphoryl enzyme intermediate where an active site serine residue is phosphorylated (Reid and Wilson, 1971). ALP acts as an indicator of cholestatic changes (Plaa and Hewitt, 1986).
Table 2.1: Effect of adriamycin (ADR) and lipoic acid (LA) on kidney enzymes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>2.30 ± 0.23</td>
<td>1.63 ± 0.18a</td>
<td>2.32 ± 0.20</td>
<td>2.28 ± 0.21d</td>
</tr>
<tr>
<td>ACP</td>
<td>0.15 ± 0.016</td>
<td>0.13 ± 0.017</td>
<td>0.17 ± 0.021</td>
<td>0.14 ± 0.019</td>
</tr>
<tr>
<td>LDH</td>
<td>0.44 ± 0.04</td>
<td>0.25 ± 0.05a</td>
<td>0.46 ± 0.04</td>
<td>0.43 ± 0.05d</td>
</tr>
<tr>
<td>AST</td>
<td>0.44 ± 0.04</td>
<td>0.28 ± 0.03a</td>
<td>0.48 ± 0.04</td>
<td>0.40 ± 0.04d</td>
</tr>
<tr>
<td>ALT</td>
<td>0.18 ± 0.02</td>
<td>0.13 ± 0.01a</td>
<td>0.21 ± 0.02</td>
<td>0.20 ± 0.02d</td>
</tr>
<tr>
<td>NAG</td>
<td>0.38 ± 0.04</td>
<td>0.20 ± 0.02a</td>
<td>0.35 ± 0.03</td>
<td>0.41 ± 0.05d</td>
</tr>
<tr>
<td>β-glu</td>
<td>0.26 ± 0.03</td>
<td>0.14 ± 0.02a</td>
<td>0.27 ± 0.04</td>
<td>0.29 ± 0.03d</td>
</tr>
<tr>
<td>Cat-D</td>
<td>0.28 ± 0.02</td>
<td>0.15 ± 0.01a</td>
<td>0.30 ± 0.03</td>
<td>0.26 ± 0.03d</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as follows: Group I - Control; Group II - ADR (7.5 mg per kg body weight, i.v); Group III - Control rats treated with LA (75 mg per kg body weight, i.p); Group IV - Rats Pretreated with LA.

One unit of enzyme activity is expressed as: ALP, ACP - μ moles x 10^-1 of phenol; LDH - μ moles of pyruvate; ALT, AST - μ moles x 10^-1 of pyruvate; NAG - μ moles x 10^-2 of p-nitrophenol; formed per min per mg protein at 37°C; β-Glu - n moles of paranitrophenol; Cat-D - μ moles of tyrosine liberated per hour per mg protein at 37°C.

Comparisons were made between *groups I and II; †groups I and III; ‡groups I and IV and ‡groups II and IV.

Values are statistically significant at *p<0.05.
There was a significant (p<0.05) reduction in the activity of ALP in the kidney of group II animals, as reported earlier by Raguenez - Viotte et al. (1988). LA supplementation was effective in restoring the activity of ALP to near normal.

LDH, a cytosolic enzyme, is a regulator of many biochemical reactions in body tissues and fluids. This enzyme has two types of subunits designated as heart type (H) and muscle type (M) (Cahn et al., 1962). The activity of LDH was significantly (p<0.05) decreased during ADR administration, indicating tissue damage. But it was observed to be near normal upon pretreatment with LA.

Aminotransferases (aspartate and alanine) being an important class of enzyme linking carbohydrate and amino acid metabolism, have established a relationship between the intermediates of the citric acid cycle (Liss et al., 1985). Activities of AST and ALT were found to be significantly decreased (p<0.05) in the kidney of ADR injected animals (Group II) when compared to the control animals (Group I). The observed decrease in the activities of the membrane bound enzymes may be due to the altered membrane permeability induced by ADR.

Enzyme efflux from dying cells depend on the development of membrane defects large enough to allow passive loss of the enzyme. Observations by Van Bambeke (1993) indicated alterations in membrane fluidity and the permeability by aminoglycosidic antibiotics with a decrease in enzyme activities which the present observations well corroborated. Significant decline in the activities of transaminases was also reported by Raguenez-Viotte et al. (1988) in hepatic tissue.
NAG is produced by tubular cells and released into the urine when they are injured. It is extensively used as a marker of tubular toxicity (Guder and Hoffman, 1992). NAG is a large protein (>125,000 daltons) and is not present in the serum in sufficient concentration to suggest that its presence in the urine is due to glomerular filtration. Observation of a significant decline (p<0.05) in the NAG activity in group II animals suggests the tubular damaging effect of ADR and the concomitant increase in NAG activity in the urine observed in the present study (Figure 2.4b).

Cat-D, a endoproteinase was found to decline significantly (p<0.05) during ADR administration. This enzyme plays an essential role in normal cellular functions and has been implicated in a variety of muscle-wasting conditions. A similar decrease in the activity of Cat-D has been reported in skeletal muscles of rats administered with ADR (Zima et al., 2001).

ADR is reported to accumulate more in the kidney (Muhammed and Kurup, 1983) than in any other organs. β-Glu, a lysosomal marker showed a marked decline (p<0.05) in the renal tissue of rats administered ADR. A similar decrease in the activity of β-Glu has been reported in the cardiac tissue indicating lysosomal damage (Geetha, 1993).

Relative to the administration of ADR, pretreatment with LA reduced the untoward effects of ADR normalizing these enzyme activities (p<0.05), suggesting the membrane stabilizing effect of LA.
2.3.2 Nucleic acids

The effect of ADR and LA administrations on the nucleic acid content of the kidney are tabulated in table 2.2. ADR administration was not found to result any significant alterations in the RNA content of the kidney. However, a significant reduction (P<0.05) was observed in the levels of DNA content which were maintained on LA pretreatment.

Krieglstein et al. (1992) hypothesised that due to oxidation of proteins and nucleic acids, the free radicals by themselves lead to the loss of membrane integrity and cell function and eventually cell death. The biological efficacy of DHLA in lowering DNA damage was brought to lime light by Sies et al. (1991). Furthermore, work by Devasagayam et al. (1993) established the fact that among the several biologically and pharmacologically active sulfur compounds examined, only LA and DHLA provided protection to plasmid DNA against singlet molecular oxygen. The observations made in the present study corroborate the fact that LA affords protection to the cellular proteins and nucleic acids by combating the oxidative stress.

2.3.3 Tissue glycolytic enzymes

Table 2.3 shows the effect of acute ADR administration on certain glycolytic enzymes of the tissue. Activities of renal glycolytic enzymes except aldolase remained unaltered in the rats administered ADR. Aldolase, which is responsible for splitting fructose-1,6-diphosphate into two trioses, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (de Duve et al., 1962) showed a significant decline (p<0.05) on ADR injection. The insulin like action of
Table 2.2: Effect of adriamycin (ADR) and lipoic acid (LA) on nucleic acid content in kidney

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>8.25 ± 0.66</td>
<td>6.98 ± 0.80a*</td>
<td>8.26 ± 0.57</td>
<td>8.23 ± 0.66d*</td>
</tr>
<tr>
<td>RNA</td>
<td>6.32 ± 0.69</td>
<td>6.29 ± 0.60</td>
<td>6.35 ± 0.73</td>
<td>6.30 ± 0.69</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1

DNA and RNA - mg per g wet tissue.

Comparisons were made between *groups I and II; *groups I and III; *groups I and IV and *groups II and IV.

Values are statistically significant at *p<0.05.
EFFECT OF ADR AND LA ON CARBOHYDRATE METABOLISM

Glyceraldehyde 3-phosphate dehydrogenase

Fructose-6-phosphate

Phosphofructokinase

Fructose-1,6-bisphosphate

Fructose-1,6-diphosphatase

Glyceroldehyde 3-phosphate dehydrogenase

1,3-bisphosphoglycerate

Phosphoglycerate kinase

3-phosphoglycerate

Phosphoglycerate mutase

2-phosphoglycerate

Enolase

Phosphoenolpyruvate

Pyruvate

Pyruvate dehydrogenase

Acetyl-CoA

Citrate synthase

Citrate

Aconitase

Isocitrate

α-ketoglutarate dehydrogenase

α-ketoglutarate

Succinyl-CoA synthetase

Succinyl-CoA

Succinyl-CoA dehydrogenase

Succinate

Fumarase

Malate

Malate dehydrogenase

Oxaloacetate

Sited of action of ADR

Sited of action of LA

Inhibition

Activation

ADR

LA
Table 2.3: Effect of adriamycin (ADR) and lipoic acid (LA) on certain glycolytic enzymes in kidney

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK</td>
<td>8.86 ± 0.79</td>
<td>8.80 ± 0.96</td>
<td>8.88 ± 0.88</td>
<td>8.82 ± 0.97</td>
</tr>
<tr>
<td>PGI</td>
<td>14.30 ± 1.29</td>
<td>14.07 ± 1.13</td>
<td>14.37 ± 1.43</td>
<td>14.20 ± 1.58</td>
</tr>
<tr>
<td>Aldolase</td>
<td>10.53 ± 0.94</td>
<td>9.16 ± 0.95a*</td>
<td>10.58 ± 0.85</td>
<td>10.48 ± 1.04d</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1.

One unit of enzyme activity is expressed as: HK - n moles of glucose - 6-phosphate; PGI - n moles of fructose; aldolase - n moles of glyceraldehyde; formed per min per mg protein.

Comparisons were made between a groups I and II; b groups I and III; c groups I and IV and d groups II and IV.

Values are statistically significant at *p<0.05.
LA (Gandhi et al., 1985) might be responsible for the increase in the glycolytic enzyme (aldolase) in group IV rats. A similar observation made in our laboratory with LA on gentamicin induced glycolytic changes lend support to the results of the present study (Sandhya et al., 1995).

2.3.4 Tissue gluconeogenic enzymes

Changes pertaining to the renal gluconeogenic enzymes (G6P, FDP) due to ADR are presented in table 2.4. G6P is a typical microsomal enzyme and greatly prevails in the gluconeogenic organs such as liver and kidney. It is a multifunctional enzyme which possess several hydrolytic and synthetic activities besides the splitting of glucose-6-phosphate and occupies an important and sensitive position in carbohydrate metabolism. FDP is another key enzyme that regulates gluconeogenesis and is seen located in the soluble fraction of cells. In the present study, no noticeable change was observed in the activities of gluconeogenic enzymes upon acute ADR administration.

2.3.5 TCA cycle enzymes

The activities of TCA cycle enzymes in the kidney of ADR injected rats is presented in table 2.5. There was a decline (p<0.05) in the activities of ICDH and MDH which were restored to near normal levels on LA pretreatment (Group IV). However no significant alteration was noticed in the activity of SDH.

The TCA cycle plays a major role in the oxidation of carbohydrates. Carbon dioxide formed in one turn of the TCA cycle is derived from, oxaloacetate and thus there is 'a crossing over' at oxaloacetate between glucose oxidation and
Table 2.4: Effect of Adriamycin (ADR) and lipoic acid (LA) on certain gluconeogenic enzymes in kidney

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6P</td>
<td>27.11 ± 2.43</td>
<td>27.08 ± 2.16</td>
<td>26.93 ± 2.69</td>
<td>26.90 ± 2.95</td>
</tr>
<tr>
<td>FDP</td>
<td>22.36 ± 2.23</td>
<td>21.98 ± 1.97</td>
<td>20.01 ± 2.40</td>
<td>19.96 ± 2.10</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1.

One unit of enzyme activity is expressed as: G6P and FDP - n moles of phosphorous oxidized per min per mg protein.

Comparisons were made between §groups I and II; §groups I and III; §groups I and IV and §groups II and IV.

Values are statistically significant at *p<0.05.
Table 2.5: Effect of adriamycin (ADR) and lipoic acid (LA) on TCA cycle enzymes in kidney

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICDH</td>
<td>5.82 ± 0.64</td>
<td>2.13 ± 0.19a</td>
<td>5.89 ± 0.70</td>
<td>5.79 ± 0.72d'</td>
</tr>
<tr>
<td>SDH</td>
<td>18.79 ± 2.25</td>
<td>17.84 ± 1.51</td>
<td>19.34 ± 2.12</td>
<td>18.71 ± 1.88</td>
</tr>
<tr>
<td>MDH</td>
<td>5.87 ± 0.46</td>
<td>2.36 ± 0.26a</td>
<td>5.96 ± 0.54</td>
<td>5.83 ± 0.60d'</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1.

One unit of enzyme activity as: ICDH - n moles of α-ketoglutarate formed; SDH - μ moles of succinate formed; MDH - μ moles of NADH oxidised; per min per mg protein.

Comparisons were made between a groups I and II; b groups I and III; c groups I and IV and d groups II and IV.

Values are statistically significant at *p<0.05.
glucose formation that ADR has the tremendous ability to inhibit oxidative phosphorylation (Muhammed and Kurup, 1982) is substantiated by the results of the present study.

2.3.6 Tissue phosphohydrolases

Effect of ADR and LA on phosphohydrolases in kidney is highlighted in table 2.6. The major function of the kidney is the net transport of sodium and other solutes across the tubular epithelium (Figure 2.2). The hydrolysis of ATP occurs as a result of the interaction of ions with ion-specific ATPases that are present in renal tubular cells. The energy available is utilized for the coupled transport of other solutes (Martinez and Schwartz, 1976).

Na\(^+\), K\(^+\)-ATPase is an integral membrane protein that catalyses the active transport of Na\(^+\) and K\(^+\) across the membrane of eukaryotic cell. The activity is regulated by intracellular net concentrations of G-protein and second messengers. The distribution of Na\(^+\), K\(^+\)-ATPase among the zones of kidney may reflect differences in sodium transport activities along the length of the nephron. Net tubular reabsorption of sodium by the mammalian kidney varies directly with renal Na\(^+\), K\(^+\)-ATPase activity and the tubular transport of sodium correlates with the Na\(^+\), K\(^+\)-ATPase activity in discrete segments of the nephron (Shelko et al., 1976).

The transport mechanism for calcium regulates the steady state intracellular concentration of calcium. This is directly coupled to a specific Ca\(^{2+}\) activated ATPase present in the tubular cells. In the presence of calcium the renal Ca\(^{2+}\)-ATPase hydrolyses GTP, ITP and UTP in addition to ATP (Cohen and Kamm,
Table 2.6: Effect of adriamycin (ADR) and lipoic acid (LA) on phosphohydrolases in kidney

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, K⁺ - ATPase</td>
<td>0.50 ± 0.04</td>
<td>0.38 ± 0.03a*</td>
<td>0.51 ± 0.05</td>
<td>0.43 ± 0.06d*</td>
</tr>
<tr>
<td>Mg²⁺ - ATPase</td>
<td>0.55 ± 0.06</td>
<td>0.53 ± 0.06</td>
<td>0.58 ± 0.07</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>Ca²⁺ - ATPase</td>
<td>0.36 ± 0.03</td>
<td>0.21 ± 0.02a*</td>
<td>0.40 ± 0.04</td>
<td>0.33 ± 0.04d*</td>
</tr>
<tr>
<td>Total ATPase</td>
<td>1.44 ± 0.14</td>
<td>1.10 ± 0.09a*</td>
<td>1.46 ± 0.15</td>
<td>1.38 ± 0.16d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1.

One unit of enzyme activity is expressed as: Na⁺, K⁺ - ATPase, Mg²⁺ - ATPase, Ca²⁺ - ATPase - μ moles of phosphorous formed per min per mg protein.

Comparisons were made between a groups I and II; b groups I and III; c groups I and IV and d groups II and IV.

Values are statistically significant at *p<0.05.
Figure 2.2 Schematic representation of proximal tubular cell, indicating functional relationships between intracellular ATP concentration and $\alpha$-MG uptake. ATP is the driving force for the Na$^+$/K$^+$-ATPase which creates a sodium gradient across the cell membrane allowing Na$^+$/glucose cotransport to proceed as a secondary active transport process (Boogaard et al., 1989).
1981a) thereby regulating the activity of Mg$^{2+}$ and Na$^+$, K$^+$ - ATPases. Therefore, Ca$^{2+}$ ATPase may play a role in the regulation of sodium reabsorption.

Mg$^{2+}$ activated ATPase is distributed in all renal cell compartments. The Mg$^{2+}$ ion functions to form Mg$^{2+}$-ATP complex which is the substrate for the enzyme. Mg$^{2+}$-ATPase plays a role in endergonic processes other than ion transport and it utilizes a pool of ATP that is not directly related to the change in free energy for sodium transport (Cohen and Kamm, 1981b).

ADR administration lead to a decrease in the activities of Na$^+$, K$^+$, ATPase and Ca$^{2+}$ - ATPase in the kidney (p<0.05). Our results are in consonance with that of Geetha and Shyamala Devi (1992) who has reported a decline in the activities of ATPases in cardiac tissue of rats receiving ADR. Pretreatment with LA (Group IV) maintained the levels of ATPases to nearly normal, thus showing LA to be an effective nephroprotectant. Acute ADR administration had no significant effect on Mg$^{2+}$-ATPase.

2.3.7 Tissue lipid peroxidation (LPO)

A free radical may be defined as any species capable of independent existence and possessing one or more unpaired electrons. These radicals can react with other molecules in many ways. The net effect is that the radical donates its unpaired electron to another molecule making it a radical. Potential sources of oxidants include the mitochondrial electron transport chain components, the endoplasmic and nuclear membrane electron transport systems, the prostaglandin synthetase and lipoxygenase systems, soluble enzymes, proteins, endogenous
Fig 2.3 Effect of ADR and LA on LPO in the renal tissue

Values represent the mean ± SD for six rats
Comparisons were made between a-groups I and II, b-groups I and III, c-groups I and IV and d-groups II and IV
Values are statistically significant at *P < 0.05
autooxidizable compounds, xenobiotics and their metabolites (Freeman and Crapo, 1982).

An increased concentration of the end products of LPO is the evidence most frequently quoted for the involvement of free radicals in human disease (Halliwell and Chirico, 1993). Lipid peroxides not only play an important role in the genesis of many chronic health problems such as cardiovascular diseases, cancer and diabetes, but also evoke adverse effects on the normal organs into which they become distributed (Yagi, 1987).

LPO on membranes leads to the oxidation of thiol groups of proteins, decrease in the relative content of polyunsaturated fatty acids and changes in the membrane receptor structure and function (Davies and Slater, 1988). The enzymatically induced LPO is found to operate in microsomes (Ernster and Nordenbrand, 1967) and mitochondria (Klimek, 1988). During the initiation of the process of LPO, Fe$^{3+}$ is converted to Fe$^{2+}$. The non-enzymatic LPO is mediated by ascorbate and Fe$^{2+}$. Ascorbate stimulates Fe$^{2+}$ induced LPO by the regeneration of Fe$^{2+}$ from Fe$^{3+}$ (Bast et al., 1987).

Transition metals (Fe$^{2+}$ or Cu$^{+}$) react with H$_2$O$_2$ to form $^•$OH radical, known as Fentons reaction. Transition metals thus play an important role in the formation of $^•$OH (Halliwell, 1999). Transition metals may be released from proteins such as ferritin (Harris et al., 1994) and the (4Fe-4S) center of different dehydrases by reactions with O$_2$$^-$. This mechanism specific for living cells has been called the in vivo Haber - Weiss reaction (Fridovich, 1997). Ascorbic acid also shows prooxidant properties. Mixtures of copper and iron react with ascorbic acid to stimulate LPO and formation of $^•$OH from H$_2$O$_2$ (Halliwell, 1990).
Figure 2.3 represents the level of MDA, an end product of LPO, measured in the kidney homogenate of control and experimental animals. Incubation of the homogenate with ferrous sulphate and ascorbate increased (p<0.05) the malondialdehyde formation in the case of group II rats. Whereas LA pretreatment brought down the MDA levels to nearly that of control animals.

\( \text{H}_2\text{O}_2 \) reacts with ADR semiquinone to generate the extremely reactive \(^{\cdot}\text{OH} \) which participates in the peroxidation of lipids leading to increased malondialdehyde formation (Mimnaugh et al., 1985). Increase in LPO following ADR administration as observed in the present study has been reported earlier by Ciaccio et al., 1993; Gorgun et al., 1999 and Montilla et al., 2000.

Thiols are thought to play a pivotal role in protecting cells against LPO (Pryor, 1973). LA, a ‘universal antioxidant’ has been reported to be effective in scavenging \(^{\cdot}\text{OH} \) generated by Fenton - type reactions (Haenen et al., 1989) and also a scavenger of peroxide and \( \text{O}_2^{\cdot}\) (Packer et al., 1995a) thus rendering protection against ADR induced LPO.

### 2.3.8 Tissue antioxidants

There was significant reduction in all the non-enzymatic antioxidants (GSH, Vit E, Vit C) except Vit A in ADR administered rats (Table 2.7). GSH (\( \gamma\)-L-glutamyl L-cysteinyl - glycine) is a cysteine containing tripeptide and the most abundant non-protein thiol in mammalian cell. GSH, has been proved to play a crucial role in some other important biochemical processes as well, including a great number of bioreductive reactions, transports and the detoxification of different xenobiotics (Penninckx and Elskens, 1993).
### Table 2.7: Effect of Adriamycin (ADR) and Lipoic Acid (LA) on Kidney Antioxidants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>21.30 ± 1.91</td>
<td>16.38 ± 1.62a*</td>
<td>21.06 ± 2.31</td>
<td>21.21 ± 1.69d*</td>
</tr>
<tr>
<td>Vit E</td>
<td>0.60 ± 0.05</td>
<td>0.48 ± 0.06a*</td>
<td>0.62 ± 0.07</td>
<td>0.57 ± 0.06d*</td>
</tr>
<tr>
<td>Vit C</td>
<td>1.01 ± 0.09</td>
<td>0.90 ± 0.07a*</td>
<td>1.04 ± 0.10</td>
<td>0.99 ± 0.07d*</td>
</tr>
<tr>
<td>Vit A</td>
<td>0.62 ± 0.06</td>
<td>0.57 ± 0.07</td>
<td>0.66 ± 0.09</td>
<td>0.60 ± 0.08</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1.

Enzyme activities are expressed as GSH and Vit C - µg per mg protein; Vit E and Vit A - mg per g tissue.

Comparisons were made between *groups I and II; groups I and III; groups I and IV and groups II and IV.

Values are statistically significant at *p<0.05.
The GSH metabolic pathway contributes to the protection against peroxides in a complex manner. GSH-dependent enzymes have been demonstrated to be involved in the elimination of peroxides and in the protection against toxic agents released during LPO and in the restoration of altered GSH/GSSG balance of the cells (Emri et al., 1997). It is well known that GSH reduces peroxides in a non-enzymatic reaction (Meister and Anderson, 1983).

GSH exhibits its antioxidant effect by reacting with $O_2^-$ and $^{*}OH$ following the formation of GSSG (Burk, 1983). GSH biosynthesis has been shown to be influenced by the total sulphur containing aminoacid of the diet and is synthesized directly from them by the reactions of $\gamma$-glutamyl cycle (Tateishi et al., 1974). The present results are comparable to that of Venketasan et al., 2000 who has reported a decrease in the renal GSH content during ADR administration.

In the present investigation, LA pretreatment was found to be significantly (p<0.05) restore the thiols status of the cell to near normal. A direct link between the thiol status of the membrane and cellular GSH has been observed (Kosower and Kosower, 1983). Membrane sulphhydryl groups play an essential role in maintaining the structural integrity of membrane. Moreover LA takes part in cellular detoxification processes, specifically in de novo synthesis of GSH (Han et al., 1997).

Lipid soluble agents trap membrane associated radicals and provide antiperoxidative protection. Among them Vit E, is considered as the most efficient biological lipid soluble antioxidant (Bier et al., 1983). Vit E, a chain breaking antioxidant is the first antioxidant present in the cell membrane that counteracts the entry of free radicals into the cell and ultimately gets transformed to
tocopheroyl radical. Earlier reports have postulated that Vit E is an intracellular free radical scavenger which can minimize cellular peroxidation following intravenous administration of ADR (Wang et al., 1980).

Vit C also has antioxidant properties (Niki, 1991) and exhibits synergistic interaction with the tocopheroyl radical, resulting in the regeneration of tocopherol (Packer et al., 1979). The antioxidant activities of α-tocopherol is enhanced in the presence of ascorbic acid (Abbey et al., 1990).

Ascorbate imparts its protection by undergoing oxidation ultimately forming dehydroascorbate. GSH helps in the reduction of dehydroascorbate back to ascorbate and when there is a reduction in the level of GSH, this conversion is affected and hence, Vit C level is lowered. ADR induced depletion of water soluble and lipid soluble antioxidants lead to increased susceptibility of the tissues to free radical damage.

Vit A is also reported to be an effective free radical scavenger (Abbey et al., 1990). It is a quencher of singlet oxygen and has the ability to react directly with the peroxyl radicals involved in LPO and traps them for further propagation (Allard et al., 1994).

Administration of LA, a nonprotein thiol, reveals its effectiveness in affording protection to cell membrane, by a possible interaction with the non-enzymatic antioxidants namely GSH, α-tocopherol and ascorbate. LA and even more so its dihydro derivative, DHLA into which it is rapidly converted after entering cellular metabolism, might act as an antioxidant for ascorbate and α-tocopherol (Scholich et al., 1989). It can be speculated that the dithiol, apart from
effectively clearing the peroxyl, 'OH and O₂⁻ radicals, effectively enhances the levels of these antioxidants by providing the reducing milieu and regenerating them via the reduction of their radicals. The above observations highlight the antioxidant property of LA.

2.3.9 Tissue antioxidizing enzymes

The activities of various antioxidizing enzymes of the kidney are presented in table 2.8. ADR administration caused a significant decline (p<0.05) in the activities of all the antioxidizing enzymes studied other than GST.

Under normal conditions, cells can tolerate mild oxidative stress, which often results in up-regulation of the synthesis of antioxidant defense systems in an attempt to restore the balance. Cytoprotective enzymes are located within both hydrophilic and hydrophobic compartments of the cells, while the antioxidants are both intra and extracellular.

Though CAT is primarily found in the peroxisomes, a small fraction may also be present in the cytoplasm. It catalyses the conversion of H₂O₂ generated by a variety of oxidases to water and molecular oxygen. H₂O₂ can react with metal chelates via the Fenton reaction and generate 'OH, a highly reactive species which can oxidize a wide variety of organic compounds including PUFA.

Compared to control rats, CAT activity was significantly (p<0.05) depressed in the kidney of rats administered with ADR. A report that lends credence to the above fact is that of Van den Branden et al. (2000) who observed a significant decline in the activity of CAT. Inhibition of CAT activity during
Table 2.8: Effect of adriamycin (ADR) and lipoic acid (LA) on kidney antioxidising enzymes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>166.21 ± 14.95</td>
<td>143.30 ± 13.80a*</td>
<td>166.38 ± 13.98</td>
<td>166.24 ± 15.36d'</td>
</tr>
<tr>
<td>SOD</td>
<td>5.89 ± 0.59</td>
<td>3.77 ± 0.44a*</td>
<td>5.94 ± 0.63</td>
<td>5.91 ± 0.70d'</td>
</tr>
<tr>
<td>GPx</td>
<td>12.98 ± 1.03</td>
<td>9.22 ± 0.88a*</td>
<td>12.96 ± 1.10</td>
<td>13.00 ± 1.43d'</td>
</tr>
<tr>
<td>GR</td>
<td>2.18 ± 0.28</td>
<td>1.80 ± 0.27a*</td>
<td>2.19 ± 0.26</td>
<td>2.21 ± 0.13d'</td>
</tr>
<tr>
<td>GST</td>
<td>18.96 ± 2.08</td>
<td>18.41 ± 2.20</td>
<td>18.83 ± 2.07</td>
<td>18.86 ± 1.91</td>
</tr>
<tr>
<td>G6PD</td>
<td>2.04 ± 0.22</td>
<td>1.36 ± 0.12a*</td>
<td>1.98 ± 0.24</td>
<td>2.01 ± 0.26d'</td>
</tr>
<tr>
<td>y-GT</td>
<td>1.99 ± 0.18</td>
<td>1.28 ± 0.13a*</td>
<td>1.98 ± 0.22</td>
<td>2.03 ± 0.16d'</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats

Treatment of groups are as in table 2.1

Enzyme activities are expressed as CAT - μg of H₂O₂ consumed per min per mg protein; SOD - units per mg protein. (One unit is equal to the amount of enzyme required to inhibit autoxidation of pyrogallol by 50%); GPx - μg of reduced GSH utilized per min per mg protein; GR - n moles of NADPH oxidised per min per mg protein; GST - n moles of CDNB - GSH conjugate formed per min per mg protein; G6PD - n moles of NADPH formed per min per mg protein; y-GT - μ moles of paranitroaniline formed per min per mg protein at 37°C.

Comparisons were made between *groups I and II; groups I and III; groups I and IV and groups II and IV.

Values are statistically significant at *p<0.05.
oxidative stress causes accumulation of $H_2O_2$ leading to peroxidation of lipids (Rister and Bachner, 1976).

SOD was the first genuine ROS - metabolizing enzyme discovered (Mc Cord and Fridovich, 1969). In eukaryotic cells, $O_2$ can be metabolized to $H_2O_2$ by two metal containing SOD isoenzymes, an 80 KDa tetrameric Mn-SOD present in mitochondria and the cytosolic 32 - KDa dimeric Cu/Zn - SOD. In the reaction catalyzed by SOD, two molecules of $O_2$ form $H_2O_2$ and molecular oxygen and thus acts as a source of cellular $H_2O_2$.

In 1957, Mills discovered an enzyme in erythrocytes, GPx, which catalyzed the reduction of $H_2O_2$ to water using GSH as the donor substrate. GPx was later identified in other tissues and found to act on a variety of organic hydroperoxides, GPx contains selenocysteine at its active site and this enzyme is more stable to peroxide mediated damage. GPx reduces several free fatty acid hydroperoxides, t-butyl hydroperoxides and cumene hydroperoxides at rates comparable to that of $H_2O_2$ (Mc Cay et al., 1976). Action of GPx is complementary to that of Vit E. The present results are in agreement with that of Van den Branden et al., 2000 who has reported a decline in the activity of GPx in animals administered ADR.

GST are a group of selenium independent enzymes that exhibit GPx activity with fatty acid peroxides and utilize GSH as hydrogen donor (Jakoby and Habig, 1980). No significant alterations in the activity of renal GST was observed, whereas animals injected with ADR (Group II) showed a significant ($p<0.05$) decline in the activities of GR and G6PD. G6PD plays an important role in the generation of the reducing potential NADPH and it is the first and the rate
limiting enzyme in the HMP-Shunt. LA elicits G6PD activity producing more reducing equivalents and subsequently regenerating GSH from GSSG.

γ-GT is predominantly located in the renal tubular brush border and in particular the renal proximal tubule (Guder and Ross, 1984). γ-GT which facilitates the uptake of GSH and cystine to support the denovo synthesis of GSH in mammalian cells (Shi et al., 1994) was found to show a significant decrease (p<0.05) in rats injected with the antineoplastic agent, ADR. Relative to the administration of ADR the antioxidant LA guarded the renal cells by boosting the antioxidant defence armory as evidenced by significant (p<0.05) elevations in the activities of antioxidant enzymes. A decrease in γ-GT activity in renal tissue might be due to increased tubular lesion, since the brush border of the proximal tubules can be a toxic target of ADR.

During such membrane disturbances, a thiol/disulphide exchange has been observed in the presence of dihydrolipoic acid/lipoic acid (DHLA/LA) redox couple. LA may thereby stabilize membranous enzymes and contribute to the amelioration of membrane integrity in ADR challenged cell injury.

2.3.10 Protein bound carbohydrates

The influence of ADR on the level of tissue glycoproteins are presented in table 2.9. Hexose, hexosamine and sialic acid were decreased (p<0.05) in the kidney due to injection of the aminoglycosidic antibiotic. Compared to group II animals, LA pretreatment produced a mild, but significant increase (p<0.05) in the level of these protein-bound carbohydrates. ADR administration did not cause any significant change in the level of hexuronic acid.
Table 2.9: Effect of adriamycin (ADR) and lipoic acid (LA) on protein bound carbohydrates in kidney

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose</td>
<td>3.18 ± 0.35</td>
<td>2.66 ± 0.24a</td>
<td>3.21 ± 0.38</td>
<td>3.15 ± 0.32d'</td>
</tr>
<tr>
<td>Hexosamine</td>
<td>0.50 ± 0.05</td>
<td>0.36 ± 0.04a</td>
<td>0.56 ± 0.07</td>
<td>0.47 ± 0.06d'</td>
</tr>
<tr>
<td>Hexuronic acid</td>
<td>0.28 ± 0.03</td>
<td>0.26 ± 0.02</td>
<td>0.30 ± 0.03</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>27.77 ± 3.33</td>
<td>20.38 ± 2.10a</td>
<td>27.96 ± 3.63</td>
<td>27.72 ± 3.06d'</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1.

Hexose, Hexosamine, Hexuronic acid - mg per 100 mg defatted tissue; Sialic acid - µg per 100 mg defatted tissue.

Comparisons were made between agroups I and II; bgroups I and III; cgroups I and IV and dgroups II and IV.

Values are statistically significant at *p<0.05.
Glycolipids and glycoproteins are recognized as important groups of components involved in different cellular functions. They play a significant role in contributing to the surface properties of the cells. Protein bound hexose provides hydrophilic points on the cell membrane (Eylar et al., 1962) and thus participates in cation binding. The function of the membrane is also likely to be influenced by the changes in glycoprotein components with their characteristic oligosaccharide and sialic acid residues.

The various proteoglycans and glycoproteins contribute to the glomerular polyanion concentration. Decreased glomerular sialic acid level has been observed in a variety of human and experimental glomerulopathies (Timpl and Dziadeek, 1986). The depletion of the protein bound carbohydrates may possibly be attributed to the interference of the antibiotic, causing concurrent depletion of negative charges in the cell membranes.

2.3.11 Urinary constituents

Effect of ADR and LA administrations on certain urinary constituents is depicted in table 2.10. A single intravenous dose of ADR did not elicit any significant change in the levels of uric acid, creatinine and GAG's. However, marginal increase (p<0.05) in the excretion of urea, phosphorous and protein were observed in the urine of ADR administered rats (Group II). Our results are thus in line with the work of Bertani et al. (1982) who has reported increased proteinuria in rats administered with ADR.
Table 2.10: Effect of adriamycin (ADR) and lipoic acid (LA) on urinary constituents

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (ADR)</th>
<th>Group III (LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>16.01 ± 1.44</td>
<td>18.73 ± 1.81a*</td>
<td>15.94 ± 1.48</td>
<td>16.12 ± 1.75d†</td>
</tr>
<tr>
<td>Uric acid</td>
<td>6.15 ± 0.68</td>
<td>6.20 ± 0.74</td>
<td>6.11 ± 0.59</td>
<td>6.18 ± 0.66</td>
</tr>
<tr>
<td>Creatinine</td>
<td>9.82 ± 1.08</td>
<td>10.02 ± 0.90</td>
<td>9.80 ± 0.78</td>
<td>9.88 ± 0.98</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.76 ± 0.06</td>
<td>2.80 ± 0.28a*</td>
<td>0.71 ± 0.07</td>
<td>0.81 ± 0.08d†</td>
</tr>
<tr>
<td>Protein</td>
<td>16.24 ± 1.46</td>
<td>29.27 ± 2.88a*</td>
<td>16.20 ± 1.45</td>
<td>16.29 ± 1.61d†</td>
</tr>
<tr>
<td>GAG's</td>
<td>12.72 ± 1.14</td>
<td>12.80 ± 1.15</td>
<td>12.69 ± 1.14</td>
<td>12.76 ± 1.02</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 2.1.

mg per 24 hours.

Comparisons were made between a groups I and II; b groups I and III; c groups I and IV and d groups II and IV.

Values are statistically significant at *p<0.05.
Pretreatment with LA (Group IV), resulted in a significant (p<0.05) decrease in the urinary excretion of urea, phosphorous and protein when compared to that of ADR injected (group II) animals.

2.3.12 Urinary enzymes

Most of the urinary enzymes originate in the kidneys and some of them are localized in specific portions and cellular components of the nephron. Figures 2.4a and 2.4b represent the urinary excretion of some cellular marker enzymes in the treatment groups.

ALP, that serves as a marker for damage to plasma membrane, has its highest activity in the brush border membrane and in the epithelial cells lining the convoluted tubules (Scherberich et al., 1984). The isoenzymic profile of urinary ALP may aid in the diagnosis of renal disease (Cornell and Hodson, 1980). Enhanced enzyme activity could be demonstrated in rats following toxic or shock induced kidney damage and due to renal artery embolism (Donadio et al., 1986).

Mohandass et al. (1995) reported increased ALP activity following administration of a nephrotoxic agent gentamicin. Kirby and Bach (1995) reported an increase in the activity of ALP on ADR administration. The above reports lend strong support to the results of the present study where a similar and significant (P<0.05) increase in ALP activity was observed in the urine of animals (Group II). The increase in the activity of the enzyme in urine, may be due to decreased kidney ALP activity. LA supplementation, reduced the enzyme activity to near normal indicating its nephroprotective role.
Fig 2.4a  Effect of ADR and LA on certain urinary enzymes (ALP, ACP, LDH and LAP)

Bars represent the mean ± SD for six rats
ALP, ACP - u moles of phenol; LDH - u moles of pyruvate, LAP - u moles of B-naphthylamide, liberated/mg creatinine/hr
Comparisons were made between a-groups I and II, b-groups I and III, c-groups I and IV and d-groups II and IV
Values are statistically significant at *P < 0.05
LDH is mainly found in the cytoplasm of the soluble fraction of the cell. Urinary LDH activity may be derived from serum, the kidney cells and prostatic secretion. In the present study, an elevation (p<0.05) in LDH activity is noticed in ADR treated group which suggests tubular damage. The leakage of this enzyme in the urine (Group IV) was found attenuated during LA pretreatment (p<0.05).

γ-GT, an extrinsic brush border enzyme, is more deeply localized in the membrane. Determination of γ-GT proves a useful tool in the diagnosis of proximal tubular damage. Palla et al. (1985) reported an increase in γ-GT activity after the administration of aminoglycosides. The increased excretion probably means damage to the proximal tubular epithelium and shedding of brush border membrane.

In the present study also, ADR administered rats (Group IV) showed a significant increase (p<0.05) in the urinary excretion of γ-GT when compared to controls (p<0.05). LA pretreatment minimized the enzyme activity in the urine when compared to ADR administered animals (Group IV, p<0.05) indicating the membrane protective effect of the dithiol.

NAG, with a molecular wt of 1,30,000 - 1,40,000 is not usually filtered by the glomeruli. NAG excretion also occurs in minimal change nephrotic syndrome (MCNS). During nephrosis, urinary levels of NAG are high and return toward normal in remission (Kunin et al., 1974). Urinary excretion of NAG acts as a main indicator for the tubular degeneration caused by nephrotoxic agents like antibiotics, heavy metals and antiinflammatory drugs (Price, 1992). Increase in the urinary excretion of NAG and its isoenzyme level after ADR administration was observed by Badary et al. (2000).
Fig 2.4b  Effect of ADR and LA on certain urinary enzymes
(Y-GT, NAG and B-Glu)

Bars represent the mean ± SD for six rats
Y-GT- u moles of paranitroaniline, NAG-n moles of paranitrophenol, B-Glu-u moles of paranitrophenol, liberated/mg creatinine/hr
Comparisons were made between a-groups I and II, b-groups I and III, c-groups I and IV and d-groups II and IV
Values are statistically significant at *P < 0.05
Histopathological changes in the kidney upon acute administration with adriamycin.

Plate 2.1a: Group I - Section of kidney showing normal architecture (H & E x 100).

Plate 2.1b: Group II - Section of kidney showing tubular dilatation in the collecting tubules (H & E x 100).

Plate 2.1c: Group II - Section of kidney showing mild tubular epithelial damage (H & E x 100).

Plate 2.1d: Group III - Section of kidney showing normal morphology (H & E x 100).

Plate 2.1e: Group IV - Section of kidney showing almost normal architecture (H & E x 100).
β-Glu is derived from the kidneys (lysosomes of tubular cells). It has been implicated in the biosynthetic pathway of L-ascorbic acid. A significant increase in the activity of β-Glu was observed in urine of rats administered ADR. Our results are in consonance with Ginevri et al. (1993) who has observed similar increase.

Upon pretreatment with LA the activities of the lysosomal enzymes NAG and β-Glu reverted to nearly normal values (p<0.05, Group IV). However, no significant alterations were brought about in the activities of ACP and LAP in ADR injected rats (Group II).

2.3.13 Morphological analysis

Examination of paraffin sections stained with hematoxylin-eosin indicated that the ADR administered renal tissue showed tubular dilation (2.1b) and mild tubular epithelial damage (2.1c). No consistent difference was observed in control (2.1a) and LA treated (2.1d) rats. Rats pretreated with LA showed tubules which are almost normal with only a few that showed dilation (2.1e).

Accepted for publication in part in Renal Failure (2003).