Chapter 3
3. CHRONIC STUDIES

3.1 INTRODUCTION

Toxic nephropathy (nephrotoxic nephritis) is a general term used to describe any adverse functional or structural change in the kidney due to the effect of a chemical or biologic product which is inhaled, ingested, injected or absorbed and which yields toxic metabolites with an identifiable effect on the kidneys (Schreiner and Maher, 1965). Two types of kidney damage has been clearly described: tubular damage and papillary damage (Ellis et al., 1973).

Tubular necrosis is produced by a range of chemicals and 80 nephrotoxic agents have been listed (Muebreke, 1969), the majority of which damage the proximal convoluted tubule. This region of the nephron is particularly vulnerable because 75 percent of the filtered water is reabsorbed in this segment. Once a renal injury has produced a certain degree of renal damage, progressive kidney failure is often inexorable (Walser, 1990).

Observations in animal models of progressive renal disease as well as in human renal disease have led to the hypothesis that after renal damage, a maladaptive response occurs in the remaining nephrons, which results in their eventual destruction and chronic renal failure. This hypothesis has been most extensively tested and studied in the subtotal nephrectomy model in the rat, in which surgical ablation of renal tissue to produce a ‘remnant kidney’ results in the development of progressive proteinuria, progressive renal impairment and glomerulosclerosis (Hostetter et al., 1981).
The first step in the pathogenesis of aminoglycoside nephrotoxicity involves the transport of these drugs into proximal tubular cell (Pastoriza-Munoz et al., 1979) where they become concentrated (Fabre et al., 1976) and exert their toxic influence (Houghton et al., 1976). The second step involves the deleterious interaction of these agents with one or more intracellular metabolic processes, which ultimately is expressed as a depression of renal function.

Free radicals are produced in the cell as a result of the metabolism of a number of xenobiotics. Those originating from ADR probably exert their effects indirectly, by activating oxygen to ROS (Olson et al., 1981). The toxicity of oxygen has been well documented (Gotteleib, 1971), but the basis of this toxicity and of the defenses which have evolved to deal with it have not yet been adequately explored (Gifford, 1968). The realization that $O_2^-$ is a common product of the biological reduction of oxygen (McCord and Fridovich, 1968) has led to the proposal that it is one of the reasons for oxygen toxicity.

ADR, an anthracycline antibiotic in cancer chemotherapy, undergoes a one-electron reduction to a free radical, semiquinone metabolite, which in the presence of molecular oxygen is rapidly reoxidized in a process that generates reactive oxygen metabolites (Shah, 1989). In human system, this drug is usually associated with cardiomyopathy but not nephropathy. However a single intravenous injection of ADR in rats causes the nephrotic syndrome associated with morphologic and functional changes similar to those seen in 'minimal-change disease' in humans.

Only few therapies are available to successfully treat renal diseases. This deficiency remains a frustrating aspect in the practice of clinical nephrology.
Effective treatments are certainly available for a few diseases. However, no beneficial therapy is available for most glomerular or tubulointerstitial diseases. To avoid or minimize the destructive effect of oxidative stress on biological tissues when disequilibrium occurs, therapies using drugs with antioxidant properties are actively being sought. For this reason one such aspect of the injury process that involves reactive oxygen metabolites and utility of antioxidant as potential therapy was studied.

3.2 MATERIALS AND METHODS

3.2.1 Materials, animal models and sources of fine chemicals are as in sections 2.2.1, 2.2.2 and 2.2.3 respectively.

3.2.2 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 adriamycin (1 mg per kg body weight per day i.v.) through tail vein once a week for a period of 12 weeks.</td>
</tr>
<tr>
<td>III</td>
<td>Administered lipoic acid (35 mg per kg body weight per day i.p.) once a week for a period of 12 weeks.</td>
</tr>
<tr>
<td>IV</td>
<td>Administered lipoic acid 24 hours prior to the administration of adriamycin once a week for a period of 12 weeks.</td>
</tr>
</tbody>
</table>

3.2.3 Collection of rat urine, rat tissues and preparation of tissue homogenate were done as indicated in sections 2.2.5, 2.2.6 and 2.2.7 respectively.
3.3 RESULTS AND DISCUSSION

3.3.1 Body and kidney weights

Renal functional parameters: body weight and kidney weight of the experimental rats are given in table 3.1.

ADR administration caused decreased (p<0.05) weight gain in group II animals, with no salient changes with regard to the kidney weight. A similar result has been reported by Guezmes et al. (1992). This decrease in body weight gain might be due to the mobilization and utilization of fat deposits for the synthesis of glucose. The effect on growth rate may also be due to reported inhibition of the Kreb's cycle and other enzyme systems by ADR (Geetha, 1993).

In the present study LA pretreatment was effective in restoring the body weight to near normal and this beneficial role of the drug may be due to increased energetic state of the cells when treated with LA (Bereiter-Hahn et al., 1989). Moreover, Debusk and Williams (1955) observed appreciable increase in the growth rate and utilization of food in rats when minute amounts of LA was included in the diet.

No significant change in the kidney weight was observed in ADR injected animals. With LA supplementation, the tissue weights remained unaltered as compared to their basal values.

3.3.2 Tissue enzymes

Table 3.2 shows the activities of certain renal enzymes: ALP, ACP, LDH, Cat-D, AST, ALT, NAG, β-Glu, β-Gal and 5'-nucleotidase. Rats administered ADR
Table 3.1: Effect of adriamycin (ADR) and lipoic acid (LA) on body and kidney weights

<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>Change in Body weight</td>
<td>18.92± 2.08</td>
<td>14.86± 1.48a*</td>
<td>19.01± 1.70</td>
<td>18.78± 1.89d*</td>
</tr>
<tr>
<td>Kidney weight</td>
<td>0.85± 0.09</td>
<td>0.80± 0.07</td>
<td>0.87± 0.08</td>
<td>0.83± 0.08</td>
</tr>
</tbody>
</table>

Treatment of groups are as follows: Group I - Control; Group II - ADR (1 mg per kg body weight, i.v); Group III - Control rats treated with LA (35 mg per kg body weight, ip); Group IV - Rats pretreated with LA.

Values represent the mean ± standard deviation for six rats.

Change in body weight - g; kidney weight - g per 100 g body weight.

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.
Group IV showed significant increase in the activities of all these enzymes in kidney. Relative to the administration of ADR, pretreatment with LA improved the activities of these enzymes. Group IV, nearly to that of the control levels.

ALP is abundant in kidney and serves as the marker for damage to the renal proximal tubules. The explanation for decline in the enzyme activity may be due to the decreased translocation of the enzyme across the epithelial membrane or, leak of the enzyme in general circulation from the collateral circulation. Decrease in the activity of renal ALP after renal damage could be correlated with the increase in ALP activity in urine (Pollak et al., 1960). ADR is reported to bind to the anionic phospholipids (Goormaghtigh et al., 1980b) present on the brush border membrane. Hence its binding may inhibit the activity of the neighbouring ALP molecule. This report tends to support the present results, wherein a decrease in the activity of ALP was observed.

When numerous tubular cells disintegrate or when tubular permeability is disturbed, renal enzyme excretion markedly increases (Raab, 1972). ADR administration brought about a decline in the activity of ACP in the heart (Singal et al., 1988). Reduced levels (p<0.05) of ACP activity in the renal tissues during ADR administration observed in the present work, is parallel to the above observation. LA pretreatment was helpful in maintaining the activity of ACP to near normal (Group IV).

LDH is the main regulator of many biochemical reactions in the body and fluids. Cytosolic LDH is distributed over most parts of the nephron (Guder and Ross, 1984) and proved to be the most sensitive enzyme in a variety of
Table 3.2:  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>2.39 ± 0.19</td>
<td>1.50 ± 0.21a</td>
<td>2.36 ± 0.28</td>
<td>2.43 ± 0.36d</td>
</tr>
<tr>
<td>ACP</td>
<td>0.92 ± 0.08</td>
<td>0.63 ± 0.07a</td>
<td>0.95 ± 0.09</td>
<td>0.87 ± 0.08d</td>
</tr>
<tr>
<td>LDH</td>
<td>0.46 ± 0.05</td>
<td>0.38 ± 0.04a</td>
<td>0.66 ± 0.07b</td>
<td>0.55 ± 0.10d</td>
</tr>
<tr>
<td>AST</td>
<td>0.46 ± 0.05</td>
<td>0.25 ± 0.03a</td>
<td>0.50 ± 0.05</td>
<td>0.49 ± 0.04d</td>
</tr>
<tr>
<td>ALT</td>
<td>0.20 ± 0.02</td>
<td>0.11 ± 0.01a</td>
<td>0.21 ± 0.02</td>
<td>0.22 ± 0.02d</td>
</tr>
<tr>
<td>NAG</td>
<td>0.40 ± 0.04</td>
<td>0.22 ± 0.02a</td>
<td>0.38 ± 0.03</td>
<td>0.42 ± 0.04d</td>
</tr>
<tr>
<td>β-Glu</td>
<td>0.32 ± 0.04</td>
<td>0.20 ± 0.02a</td>
<td>0.33 ± 0.03</td>
<td>0.30 ± 0.03d</td>
</tr>
<tr>
<td>β-Gal</td>
<td>0.20 ± 0.02</td>
<td>0.13 ± 0.01a</td>
<td>0.22 ± 0.03</td>
<td>0.18 ± 0.02d</td>
</tr>
<tr>
<td>5' Nucleotidase</td>
<td>2.80 ± 0.33</td>
<td>1.75 ± 0.18a</td>
<td>2.83 ± 0.25</td>
<td>2.81 ± 0.33d</td>
</tr>
<tr>
<td>Cat-D</td>
<td>0.30 ± 0.03</td>
<td>0.13 ± 0.01a</td>
<td>0.32 ± 0.03</td>
<td>0.31 ± 0.04d</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.
Treatment of groups are as in table 3.1.

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 10² of p-nitrophenol; β-Glu, β-Gal - n moles of paranitrophenol; 5'-nucleotidase - µ moles of phosphorus; formed per min per mg protein; Cat-D - µ moles of tyrosine liberated per hour 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.
experimental nephropathies (Maruhn et al., 1983). Thus measurement of LDH was considered a useful tool for localization of kidney damage. LDH is functionally involved in the catalyzation of reversible steps common to both glycolysis and gluconeogenesis (Bomhard et al., 1990). LDH was found to be significantly decreased in the kidneys of ADR injected rats confirming damage to renal tissue.

Cat-D, a lysosomal endoproteinase is reported to be a glycoprotein (Takahashi et al., 1983). Glycoproteins are considered to be the susceptible target for ROS produced during LPO (Cooper et al., 1985). A report of Kim et al. (1985) says that the ROS formed during the reoxidation of ADR semiquinone radicals can damage a variety of cellular macromolecules including enzymes. Our observations are in consonance with that of Geetha, 1993; Zima et al., 2001 who has reported a decrease in the activity of cat-D in the heart tissue and skeletal muscles respectively. Activities of cat-D were maintained to near normal levels (p<0.05) upon pretreatment with LA (Group IV) indicating the nephroprotective role of the drug.

Aminotransferases (alanine, aspartate) being an important class of enzymes linking carbohydrate and amino acid metabolism, have an established relationship between the intermediates of citric acid cycle. Indicators of cell necrosis, namely AST and ALT were decreased (p<0.05) in the tissues of ADR administered rats (Group II) revealing the membranolytic characteristic of ADR, leading to altered membrane permeability, which may be responsible for the observed enzymatic variations. LA pretreatment proved useful in maintaining the enzyme activities to near normal levels in group IV animals indicating the beneficial influence of dithiol on tissue metabolism.
NAG with a molecular weight of 130,000 - 140,000 is not usually filtered by the glomeruli. NAG is one of the sensitive hydrolytic lysosomal enzymes, which is released in urine after renal tubular damage (Whiting and Brown, 1996). The decrease in NAG activity in tissue could be a result of ADR induced damage to the lysosomal membrane. Nephrotoxicity is evident with the decrease in the activity of NAG in the renal tissue and a similar decrease was observed in ADR treated rats (Group II, p<0.05).

Kidney β-Glu is localised in the lysosomes of tubular epithelial cells. Its excretion pattern in health and disease was found to be most similar to that of another lysosomal enzyme in urine, β-Gal (Davidson, 1967). The activities β-Glu and β-Gal in kidney, were seen to be significantly decreased (p<0.05) upon ADR administration (Group II). Singal et al. (1988) have also shown that the administration of ADR to rats was associated with changes in lysosomal enzyme activities. This decrease in the activities of β-Glu and β-Gal indicates lysosomal damage due to ADR (Geetha, 1993). Pretreatment with LA showed a protective effect on lysosomes thereby preventing a decline in the activities of lysosomal enzymes.

5'-nucleotidase is a plasma membrane marker enzyme which catalyses the dephosphorylation of purine 5'-monophosphate to purine nucleosides. Because nucleosides are membrane permeable, these compounds are lost from the kidney on reflow (Warnick and Lagarus, 1981) thereby substrates for purine salvage would be lost and their unavailability would limit restoration of ATP levels after an ischemic insult (Stromski et al., 1986).
Hypoxia conditions are known to stimulate the production of adenosine through catabolism of ATP. Adenosine exerts regulatory function in various organs. In kidney, it reduces the glomerular filtration rate, decreases renin release and directly regulates tubular functions (Coulson and Scheinmann, 1989). It may also play a role in the pathogenesis of some forms of acute renal failure (Bowmer et al., 1986). A significant reduction in the enzyme activity was observed during ADR therapy in rat renal tissue (p<0.05). Pretreatment with LA was found to restore the activity of 5' nucleotidase (Group IV).

3.3.3 Nucleic acids

Changes brought about in DNA and RNA contents in kidney are tabulated in table 3.3. Rats chronically administered ADR (Group II), showed a reduction (p<0.05) in the content of DNA and RNA, which were reverted to nearly normal on pretreatment with LA.

3.3.4 Tissue glycolytic enzymes

The kidney maintains body fluids and electrolyte balance by regulating the excretion of ions, salts, metabolic waste materials and water into the urine. Kidney cells therefore, require energy to maintain these functions and the primary energy donor is ATP. The overall chemical reactions that lead to the ATP synthesis have been well documented (Weinberg and Humes, 1986).

Ross et al. (1986) have demonstrated that the ATP supply is controlled by the rate of substrate cycling, or by the pathways between fructose-6-phosphate and fructose 1,6-diphosphatase in a tissue possibly with the capacity for glycolysis.
Table 3.3: 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>8.28 ± 0.66</td>
<td>7.11 ± 0.81a*</td>
<td>8.30 ± 0.74</td>
<td>8.29 ± 0.91d*</td>
</tr>
<tr>
<td>RNA</td>
<td>6.43 ± 0.58</td>
<td>5.06 ± 0.55a*</td>
<td>6.41 ± 0.51</td>
<td>6.40 ± 0.70d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

DNA, RNA - mg per g wet tissue.

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.
### Table 3.4: 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 (ADR + LA)</th>
<th>Group IV (ADR + LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK</td>
<td>8.89 ± 1.06</td>
<td>5.95 ± 0.83a</td>
<td>15.62 ± 1.66b</td>
<td>9.56 ± 0.86d</td>
</tr>
<tr>
<td>PGI</td>
<td>14.36 ± 2.08</td>
<td>10.12 ± 1.31a</td>
<td>20.36 ± 2.31b</td>
<td>14.18 ± 1.52d</td>
</tr>
<tr>
<td>Aldolase</td>
<td>10.61 ± 1.47</td>
<td>8.21 ± 0.83a</td>
<td>14.06 ± 1.61b</td>
<td>11.68 ± 1.68d</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

One unit of enzyme activity is expressed as: HK - n moles of glucose-6-phosphate; PGI - n moles of fructose-1,6-bisphosphate per min per mg protein.

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.
and gluconeogenesis. In the rat nephron, the major enzymes for glucose metabolism are Hexokinase, phosphofructokinase, pyruvate kinase and pyruvate dehydrogenase.

The effects of ADR and LA administrations on certain key glycolytic enzymes are shown in table 3.4. ADR administration brought about a significant decline (p<0.05) in the activities of glycolytic enzymes viz., HK, PGI and aldolase. Studies on the model membrane suggests that ADR exhibits specific affinity to membrane lipid domains (Duarte-Karim et al., 1976) and modifies the lipid thermotropic properties (Goldman et al., 1978). This may be a reason for ADR to inhibit D-glucose transport across the renal medulla, by interacting with the anionic phospholipids of the membrane. Our results corroborate with the work of Takahashi et al. (1987) who has reported a similar finding in gentamicin treated rats.

ROS may also contribute to a decrease rate of glycolysis. ROS increases DNA strand breaks with subsequent activation of poly-ADP-ribose polymerase activity, which contributes to NAD consumption. Consequently rate of glycolysis and the cellular energy supply is limited and the antioxidative capacity is also reduced (Spragg, 1991). High concentrations of H₂O₂ can inactivate the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase in mammalian cells (Hyslop et al., 1988). This inhibition in the transport of glucose by ADR might cause a reduction in the activity of glycolytic enzymes in the kidney.

The stimulatory effect of LA on the glycolytic enzymes might be due to the reported acceleration of glucose transport by LA (Singh and Bowman, 1970). This can be attributed to the fact that the reduction of LA to dihydrolipoate in the
intracellular milieu reduces the NADH/NAD\(^+\) ratio, leading to a decrease in the intracellular levels of ATP. This change in the nucleotide status triggers the elevation of glucose transport (Bashan et al., 1993).

The protective action may be attributed to the primary reduction of acetyl-CoA in the tissue, which eventually leads to decreased citrate levels, and which in turn leads to the activation of phosphofructokinase and hence increased glycolysis (McCarty, 1981b). Furthermore LA is reported to have an insulin like action as well (Gandhi et al., 1985).

3.3.5 **Tissue gluconeogenic enzymes**

Table 3.5 depicts the significant reduction in the activity (p<0.05) of gluconeogenic enzymes (G6P, FDP) in ADR administered rats (Group II) and accentration of the effects upon LA pretreatment. The mechanism by which ADR causes a decrease in the activity of gluconeogenic enzymes is not well known. Goldstein et al. (1987) observed a decrease in gluconeogenesis with cephaloridine, a potent nephrotoxin, due to the inhibition of glucose-6-phosphatase activity. The added inhibitory effect noted upon LA administration can be explained. This might be due to the antagonistic action of LA on gluconeogenesis. The sequestration of co-enzyme A as lipoyl co-enzyme A lowers the concentration of acetyl-CoA thus impairing gluconeogenesis by inhibiting the action of pyruvate carboxylase Bluementhal et al. (1984). Khamaisi et al. (1999) reported that LA inhibited the *in vivo* gluconeogenesis flux from alanine. Though there was a decrease in gluconeogenic enzymes upon LA pretreatment, no serious complication was noticed during the course of the experiment.
Table 3.5: 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>29.56 ± 2.06</td>
<td>25.32 ± 1.50a*</td>
<td>17.31 ± 1.20b*</td>
<td>12.80 ± 1.15c*d'</td>
</tr>
<tr>
<td>FDP</td>
<td>24.30 ± 2.43</td>
<td>18.39 ± 1.47a*</td>
<td>15.26 ± 1.37b*</td>
<td>15.16 ± 1.88c*d'</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

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.
3.3.6 Glycogen and glycogen phosphorylase

The activities of glycogen and glycogen phosphorylase in kidney of ADR administered animals is highlighted in table 3.6.

The glycogen content was decreased significantly (p<0.05), whereas the activity of glycogen phosphorylase was found to increase (p<0.05) in kidney of ADR administered animals (Group II). The decrease in glycogen content in the tissue might be a result of increased glycogen phosphorylase activity. This is in good agreement with the findings of Lietz and Bryla (1990).

A decrease in the activity of glycolytic and gluconeogenic enzymes was observed in ADR administered rats which might be the reason for the increased activity of glycogen phosphorylase as a result of which there is an increased mobilization of glycogen to meet the energy demands with a consequent reduction in glycogen content.

Pretreatment with LA increased the glycogen content and decreased the activity of glycogen phosphorylase in the renal tissue. Wagh et al. (1987) have reported that LA administration increased the glycogen content of the liver in alloxan induced diabetic condition.

3.3.7 TCA cycle enzymes of kidney

There was a marked decline (p<0.05) in the activities of MDH, SDH and ICDH (table 3.7) on ADR administration (Group II). Relative to the administration of ADR, pretreatment with LA brought about a significant (p<0.05) elevation in the activities of TCA cycle 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>Glycogen</td>
<td>4.90 ± 0.53</td>
<td>3.49 ± 0.35a</td>
<td></td>
<td>4.87 ± 0.44d</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>0.48 ± 0.04</td>
<td>0.39 ± 0.09a</td>
<td>0.45 ± 0.05</td>
<td>0.50 ± 0.06d</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Values are statistically significant at *p<0.05.

Comparisons were made between groups I and II, groups I and III, groups I and IV, and groups II and IV.

Treatment of groups are as in table 3.1.

Glycogen - mg per g wet tissue; Glycogen phosphorylase - μ moles of Pi liberated per min per mg protein.
It is well established that certain enzymes located in the mitochondria catalyse the oxidation of a number of substrates via, the citric acid cycle yielding reducing equivalents. These reducing equivalents are channeled through the respiratory chain which ultimately results in the synthesis of ATP by oxidative phosphorylation, which provides the energy needed for many cellular functions.

Reduction in the activities of TCA cycle enzymes proves the defect in aerobic oxidation of pyruvate which might lead lowered production of ATP molecules. TCA cycle enzymes which are located in the outer membrane of mitochondria, could have been affected by the free radicals produced by ADR (Geetha and Shyamala Devi, 1992). The inhibition of these enzymes may affect mitochondrial substrate oxidation and the electron transport chain, resulting in reduced oxidation of succinate and reduced rate of transfer of reducing equivalents to molecular oxygen (Rao, 1983).

The mitochondria have the ability to accumulate the drug in vivo. The accumulation of the drug in the kidney, its concentration in the kidney nuclei and mitochondria is much higher than observed in the heart. The antineoplastic drug also has tremendous ability to inhibit oxidative phosphorylation with may account for the impairment of energy metabolism of the cell (Muhammad and Kurup, 1982). Mitochondrial degeneration and dysfunction may also contribute to the pathogenesis of aminoglycoside nephrotoxicity. This might be the reason for the decrease in the activity of TCA cycle enzymes recorded in the present study.
Table 3.7: Effect of adriamycin (ADR) and lipoic acid (LA) on certain 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>5.89 ± 0.71</td>
<td>2.03 ± 0.22a*</td>
<td>6.02 ± 0.60</td>
<td>5.82 ± 0.64d*</td>
</tr>
<tr>
<td>SDH</td>
<td>19.06 ± 2.09</td>
<td>13.96 ± 1.25a*</td>
<td>20.13 ± 1.41</td>
<td>18.91 ± 2.69d*</td>
</tr>
<tr>
<td>MDH</td>
<td>5.91 ± 0.71</td>
<td>1.32 ± 0.13a*</td>
<td>6.11 ± 0.80</td>
<td>6.03 ± 0.90d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

One unit of enzyme activity is expressed as: ICDH - n moles of α-ketoglutarate formed; SDH - μ moles of succinate formed; MDH - n moles of NADH oxidised; 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.
3.3.8 Tissue phosphohydrolases

Effects of ADR and LA administrations on the activities of certain ATPases is evident from table 3.8. ATPases are membrane-bound and are mostly present on the basolateral membrane. Na\(^+\), K\(^+\)-ATPase is widely considered to be the enzyme system of the plasma membrane responsible for the active transport of Na\(^+\) and K\(^+\) (Schwartz et al., 1965). Na\(^+\), K\(^+\)-ATPase activity pumps Na\(^+\) out of the cell. As a result, the intracellular concentration of Na\(^+\) is lowered and an inward proton gradient due to Na\(^+\)-H\(^+\) exchange is established across the brush border membrane which splits up the ATP for energy purpose. In the present study a significant decrease (p<0.05) in Na\(^+\), K\(^+\), ATPase activity was observed in the kidney of ADR administered group. This observation is in agreement with the earlier report of Gabriela et al. (1998) who reported a decrease in Na\(^+\), K\(^+\)-ATPase activity in the cardiac tissue of ADR administered rats.

The Ca\(^{2+}\)-ATPase is bound to the plasma membrane in the rat proximal tubular epithelial cell. The activity of Ca\(^{2+}\) - ATPase significantly declined (p<0.05) in the renal tissues of ADR injected animals (Group II).

Mg\(^{2+}\)-ATPase is distributed in all renal cell compartments and plays a role in endergonic processes other than ion transport. The ion sensitive ATPases are poised to regulate the flow of potential energy from the mitochondria and from the cytoplasm. In the present study, Mg\(^{2+}\) - ATPase activity was significantly inhibited (p<0.05) in the kidney of animals receiving ADR injections.

Activities of phosphohydrolases were maintained at near normal levels upon pretreatment with LA (Group IV).
Table 3.8: 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>0.56 ± 0.04</td>
<td>0.34 ± 0.04(^a)*</td>
<td>0.58 ± 0.05</td>
<td>0.61 ± 0.06(^d)*</td>
</tr>
<tr>
<td>Ca(^{2+}) - ATPase</td>
<td>0.40 ± 0.03</td>
<td>0.18 ± 0.01(^a)*</td>
<td>0.41 ± 0.04</td>
<td>0.43 ± 0.05(^d)*</td>
</tr>
<tr>
<td>Mg(^{2+}) - ATPase</td>
<td>0.60 ± 0.05</td>
<td>0.24 ± 0.38(^a)*</td>
<td>0.62 ± 0.05</td>
<td>0.68 ± 0.09(^d)*</td>
</tr>
<tr>
<td>Total ATPase</td>
<td>1.58 ± 0.16</td>
<td>0.75 ± 0.07(^a)*</td>
<td>1.60 ± 0.17</td>
<td>1.70 ± 0.13(^d)*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

Na\(^+\), K\(^+\) - ATPase, Mg\(^{2+}\) - ATPase, Ca\(^{2+}\) - ATPase - μ moles of Pi 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.
3.3.9 Tissue lipid peroxidation

Oxygen is essential for the survival of aerobic cells, but it has long been known to be toxic to them when supplied at concentrations greater than those in normal air (Halliwell, 1981). The biochemical mechanisms responsible for \( \text{O}_2 \) toxicity include LPO and the generation of \( \text{H}_2\text{O}_2 \) plus the \( \text{O}_2^- \). In biochemical systems, \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) react together to form the \( \cdot\text{OH} \), which can attack and destroy almost all known biomolecules (Fridovich, 1975).

Neither LPO nor generation of \( \cdot\text{OH} \) from \( \text{O}_2^- \) occurs \textit{in vitro}, unless traces of transition metal ions, especially iron, are present (Willson, 1979). Traces of iron salts are present in all biological systems and any increase in the normal concentration will potentiate the toxic effects of oxygen (Halliwell, 1981).

The extent of peroxidation in the kidney during ADR administration is presented in figure 3.1. The kidney tissue was more susceptible to free radical damage both under basal (\( p<0.05 \)) and in the presence of stress inducers (\( p<0.05 \)). LA pretreatment prevented the formation of MDA in kidney (\( p<0.05 \)).

ADR toxicity is thought to be related to the formation of a semiquinone radical. Autoxidation of the semiquinone then leads to the formation of ROS such as \( \text{O}_2^- \) and \( \cdot\text{OH} \) (Goodman and Hochestein, 1977; Nohl and Jordan, 1983) and these species are considered to cause LPO and DNA damage.

The formation of free radicals as well as the accumulation of lipid peroxides in response to the treatment with ADR, has been well documented. LPO
Fig 3.1 Effect of ADR and LA on LPO in the neural 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
is recognised as one of the possible biochemical mechanisms for ADR associated side effects (Doroshow, 1983).

Mimnaugh et al. (1986) suggested that ADR mediated free radical production markedly increases LPO and that this event in vivo may be involved in the nephrotoxicity due to ADR. Treatment of rats with ADR resulted in significant (p<0.05) increase in MDA production in comparison with those levels of the control ones (Saad et al., 2001). Our results corroborated well with the above reports.

3.3.10 Tissue antioxidants

Table 3.9 represents the level of antioxidants in kidney of control and experimental animals. Compared to a decline (p<0.05) in antioxidant status during ADR administration (Group II), the antioxidant status were maintained (p<0.05) during pretreatment with the dithiol LA (Group IV).

GSH is a major mechanism for regulating intracellular free radical concentration (Flohe and Gunzler, 1976). GSH plays an important role in the detoxification of xenobiotic compounds, in the antioxidation of ROS and free radicals. Low levels of GSH were observed during increase in oxidative stress that increases the formation and efflux of GSSG (Eklow et al., 1984). Montilla et al. (2000) has reported a decline in the renal content of GSH in ADR administered animals. This observation supports the present findings where a decline in GSH levels with an increase in oxidative stress as evidenced by increased LPO have been observed. LA is reported to regenerate the GSH pool by reduction of GSSG (Packer et al., 1995a). Administration of LA helps to overcome the oxidative stress.
Table 3.9: 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>22.60 ± 2.16</td>
<td>15.65 ± 1.57a*</td>
<td>21.20 ± 1.91</td>
<td>20.50 ± 1.95d*</td>
</tr>
<tr>
<td>Vit C</td>
<td>1.05 ± 0.07</td>
<td>0.88 ± 0.08a*</td>
<td>1.07 ± 0.09</td>
<td>1.10 ± 0.09d*</td>
</tr>
<tr>
<td>Vit E</td>
<td>0.63 ± 0.07</td>
<td>0.45 ± 0.04a*</td>
<td>0.66 ± 0.08</td>
<td>0.65 ± 0.06d*</td>
</tr>
<tr>
<td>Vit A</td>
<td>0.65 ± 0.06</td>
<td>0.56 ± 0.06a*</td>
<td>0.67 ± 0.07</td>
<td>0.68 ± 0.05d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

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 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.
placed by ADR on the cell, by increasing the GSH status which in turn exhibits increased free radical scavenging property.

Ascorbic acid is water soluble and has been shown to be a major antioxidant in human plasma as well as in and even across cell membranes (Frei et al., 1990). It reduces α-tocopherol as well as peroxides and ROS such as O$_2^*$ (Buettner, 1993). The vitamin serves mainly to prevent lipid hydroperoxide formation in plasma lipoproteins by reducing α-tocopherol radicals formed upon reaction with lipid peroxyl radicals (Sies et al., 1992). Ascorbate also protects lipids in cell membranes by this mechanism. Intracellularly, in the aqueous phase, ascorbate and GSH act in concert to protect the cell from oxidative damage (Meister, 1995).

Vit E is a lipid soluble vitamin present in biological membranes. It contains a hydroxyl group by which it reacts with unpaired electrons and can reduce peroxyl radicals. It can function as a chain breaking antioxidant (Burton and Traber, 1990), interrupting the propagation of oxygen radical mechanisms. There is an increasing evidence suggesting that β-carotene functions as an important singlet oxygen and free radical scavenger (Burton and Ingold, 1984).

The scavenging activity of Vit A is enhanced at oxygen partial pressures characteristic of tissues. The free radical clearing ability of this fat soluble vitamin is due to delocalisation of an unpaired electron in the conjugated double bond system.

Suzuki et al. (1991) stated that LA and DHLA clear off free radicals both in the aqueous phase and in the hydrophobic membrane domains, directly
quenching, initiating and chain propagating peroxides. Additively, DHLA donates its reducing equivalents for the regeneration of water (GSH and Vit C) and lipid (Vit E) soluble antioxidants. The DHLA/LA redox couple has been found to exert a synergistic action on the antioxidant recycling mechanisms of natural membranes and low density lipoproteins in vitro, and in the protection against oxidative injury in vivo.

DHLA acts as a "double-edged sword", in that, it appears to interact directly with peroxyl radicals in the membrane or indirectly to reduce tocopheroxyl radicals via, a cascade mechanism involving the reduction of ascorbate, which inturn reduces tocopheroxyl radicals to tocopherol (Packer, 1992). This may be the possible reason for the rise in antioxidant levels during drug administration as indicated by the results of this investigation.

3.3.11 Tissue antioxidizing enzymes

The results presented in table 3.10 shows a significant (p<0.05) lowering in the activities of antioxidizing enzymes in the kidney of rats administered with ADR (Group II). However, the decreased levels of the antioxidizing enzymes in group II were restored to the basal levels on LA pretreatment (Group IV).

CAT of many organisms are mainly heme-containing enzymes (Aebi, 1974). The predominant subcellular localization in mammalian cells is in peroxisomes, where CAT catalyses the dismutation of H\textsubscript{2}O\textsubscript{2} to water and molecular oxygen. Rister and Bachner (1976) speculated that during oxidative stress, CAT activity decreases, H\textsubscript{2}O\textsubscript{2} accumulates and thereby more peroxidation of lipids is favoured. Thus a decrease in the activity of CAT was observed on ADR
Table 3.10: 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>175.30 ± 16.72</td>
<td>140.20 ± 12.12a*</td>
<td>174.89 ± 16.56</td>
<td>180.50 ± 15.81d*</td>
</tr>
<tr>
<td>SOD</td>
<td>6.20 ± 0.63</td>
<td>4.80 ± 0.49a*</td>
<td>6.21 ± 0.55</td>
<td>6.43 ± 0.64d*</td>
</tr>
<tr>
<td>GPx</td>
<td>15.20 ± 1.29</td>
<td>12.30 ± 1.10a*</td>
<td>14.80 ± 1.03</td>
<td>16.30 ± 1.32d*</td>
</tr>
<tr>
<td>GR</td>
<td>2.36 ± 0.20</td>
<td>1.26 ± 0.14a*</td>
<td>2.38 ± 0.17</td>
<td>2.05 ± 0.19d*</td>
</tr>
<tr>
<td>GST</td>
<td>20.34 ± 1.93</td>
<td>17.89 ± 1.71a*</td>
<td>20.78 ± 2.08</td>
<td>22.82 ± 2.10d*</td>
</tr>
<tr>
<td>G6PD</td>
<td>1.98 ± 0.15</td>
<td>1.12 ± 0.12a*</td>
<td>1.99 ± 0.16</td>
<td>1.89 ± 0.15d*</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>1.96 ± 0.22</td>
<td>2.64 ± 0.21a*</td>
<td>2.05 ± 0.16</td>
<td>2.11 ± 0.19d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

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; Xanthine oxidase - units per mg protein (1 unit = the amount of enzyme that bring a change in OD of 0.01 per min).

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.
administration that which is a consequence of oxidative stress (Montilla et al., 1997) and and the present findings of this study are in consonance with the above reports.

SOD constitutes an important link in the biological defence mechanism through disposition of endogenous cytotoxic $O_2^-$ which are deleterious to PUFA and structural proteins of plasma membrane (Fridovich, 1975). ADR administration caused a decline in the activities of SOD more in neoplastic cells (Kiyomiya et al., 2001) than normal cells. Van den Branden et al. (2000) has also reported a decline in the activity of SOD on ADR administration. Pretreatment with LA reverted the activity of SOD to near normal by increasing the GSH status which inturn exhibits increased free radical scavenging property. This results in the increase in the activity of SOD thereby preventing the deleterious effect of $O_2^-$ formed. Thus LA it may be seen that indirectly influences the activity of SOD.

There are atleast four different GPx in mammals (GPx 1-4), all of them containing selenocysteine. All the GPx may catalyse the reduction of $H_2O_2$ using GSH as substrate. They can also reduce other peroxides (eg. lipid peroxide in cell membranes) to alcohols (Ursini, 1995).

The catalytic mechanism proposed for reduction by hydroperoxides by GPx (Epp et al., 1983) involves oxidation of the active site selenolate ($Se^-$) to selenenic acid (SeOH) upon addition of one molecule of GSH. The selenenic acid is transformed to selenenyl sulfide adduct with GSH (Se-SG), which can be regenerated to the active selenolate and GSSG by addition of a second molecule of GSH. Thus in the reaction, two molecules of GSH are oxidized to GSSG that which, subsequently can be reduced by GR, the major mammalian GSSG-reducing
enzyme. Some data indicated that GPx has a high antioxidant importance under physiological conditions (Jones et al., 1981), while others place the enzymes as important only at events of oxidative stress (Kelner and Bagnell, 1990).

The activity of GPx, which is a constituent of GSH redox cycle showed decrease upon ADR administration in the present investigation. The fall in the GSH status might be responsible for the observed decrease in the activity of GPx. Inhibition of GSH metabolizing enzymes (GR, GST and G6PD) observed in the present study may partly be due to want of substrate (GSH) and also because of inhibition of their protein structure. Support for this concept comes from studies, where numerous proteins have been inhibited by decreased GSH content but have been subsequently reactivated when the concentration of GSH increased (Kosower and Kosower, 1976).

GST (in its basic form) is present in abundance in the cytosol of proximal tubular cells, but not, or only negligible, in other parts of the rat, rabbit and human nephron (Harrison et al., 1989). The GST's are a group of multifunctional proteins encoded by a multigene family that find hydrophobic substrates and catalyse the reaction of GSH with an electrophilic centre in these substrates (Habig et al., 1973). They perform functions ranging from catalyzing the detoxification of electrophilic compounds to protection against peroxidative damage (Leibau et al., 1994). The inhibition of GST activity on ADR administration was prevented upon pretreatment with LA (Group IV, p<0.05) as can be seen from table 3.10.

GR plays a major role in regenerating endogenous GSH from GSSG, thus maintaining the balance between the redox couple. This enzyme requires reducing
equivalent (NADPH) for its activity, which is provided by the action of G6PD. GR, a constituent of GSH-redox cycle was significantly decreased on ADR administration. This might be due to the reduced availability of NADPH. The decrease in G6PD activity and a fall in NADPH, might occur as a result of impaired flux of glucose-6-phosphate through the HMP shunt. LA pretreatment resulted in an increased availability of reducing equivalents (NADPH) possibly due to improved G6PD activity thereby significantly increasing the activity of GR with subsequent regeneration of the GSH pool.

Renal xanthine oxidase system provides a source of oxygen free radicals in ADR experimental nephrosis by generating uric acid from hypoxanthine and xanthine. Indeed recent evidence supports the idea that the xanthine oxidase/xanthine dehydrogenase system is activated in ADR nephrosis and contribute to the development of proteinuria. Accordingly the conversion of hypoxanthine and xanthine to uric acid increases after ADR administration and brings about the production of $O_2^{•−}$ (Ginevri et al., 1990).

The present results revealed that ADR administration resulted in a marked rise (p<0.05) in the renal xanthine oxidase activity which is in consonance with the aforementioned point. Compared to group II animals, LA pretreatment prevented a hike (p<0.05) in the activity of renal xanthine oxidase (Group IV).

### 3.3.12 Enzymes involved in glutathione metabolism

The activities of kidney $\gamma$-GCS and $\gamma$-GT are presented in table 3.11. A significant decline (p<0.05) was noticed in the activities of $\gamma$-GCS and $\gamma$-GT in
Table 3.11: Effect of adriamycin (ADR) and lipoic acid (LA) on glutathione metabolising 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>γGCS</td>
<td>61.96 ± 5.67</td>
<td>49.73 ± 5.47a*</td>
<td>62.44 ± 4.99</td>
<td>63.27 ± 6.32d*</td>
</tr>
<tr>
<td>γ-GT</td>
<td>2.05 ± 0.20</td>
<td>1.46 ± 0.12a*</td>
<td>2.06 ± 0.14</td>
<td>2.15 ± 0.19d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

One unit of enzyme activity is expressed as: γ-GT - μ moles of paranitroaniline; γ-GCS - μg of Pi released; 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.
group II animals administered ADR. On the contrary, pretreatment with LA enhanced the activities of these enzymes to near normal.

γ-GCS is the enzyme that catalyses the first and rate limiting step in GSH synthesis and γ-GT catalyses the break down of GSH (Alptekin et al., 1996). The intracellular synthesis of glutathione occurs as a result of the combined activities of γGCS and γ-GT. ADR has been reported to inhibit the activities of glycolytic, gluconeogenic and TCA cycle enzymes. As a consequence, cellulose energy supplies in the form of ATP would rapidly be consumed. Decreased activity of γ-GCS observed in the present study might be due to lack of ATP, which is required for its activity.

Decrease in γ-GT (p<0.05) activity in renal tissue might be due to increased tubular lesion, since the brush border of the proximal tubules can be toxic targets of ADR. Moreover, decrease in γ-GT might reflect decreased availability of GSH, since at low concentrations of GSH various amino acids and peptides were reported to form a dead-end inhibitor complex with the enzyme thereby inhibiting its activity (Curthoys and Hughey, 1979).

DHLA on the other hand was reported to increase ATP synthesis in mitochondria (Zimmer et al., 1991). The increased availability of energy might be the reason for improved γ-GCS activity (p<0.05) observed in group IV animals pretreated with LA as shown by the results of the present investigation. Further, LA increased γ-GT activity (p<0.05) by improving the GSH status thus maintaining normal GSH synthesis and hydrolysis.
### 3.3.13 Protein bound carbohydrates

Table 3.12 depicts the level of protein bound carbohydrates in the kidney. The levels of hexose, hexosamine, hexuronic acid and sialic acid were found to be decreased \( p<0.05 \) in group II animals. LA pretreatment restored the levels of the protein bound carbohydrates to near normal (Group IV) suggesting the renoprotective effect of the dithiol.

The glomerular capillary wall is covered by polyanionic macromolecules, which are collectively referred to as the glomerular polyanion. The glycoproteins come under this classification, and sialic acid is an important constituent of the renal glomerular polyanion. The protein bound hexose provide the hydrophilic points on the cell membrane, while sialic acid coat is responsible for the electronegativity of the membrane (Eylar *et al.*, 1962). Sialic acid coat contributes to the electrostatic selectivity of the glomerular filter (Cotran and Rennke, 1983). It is also necessary for the anchorage of endothelial and epithelial cells to the glomerular basement membrane.

Bertani *et al.*, 1982 demonstrated that depletion of sialic acid is involved in the loss of the glomerular barrier function induced by chronic ADR administration. The disappearance of sialoproteins clearly preceded both the appearance of ultrastructural changes and the onset of proteinuria. Maintenance of membrane fluidity within narrow limits is a prerequisite for proper functioning of the cell and glycoproteins play a key role in this connection. LA skillfully regulates the fluidity of the cell membranes by rendering protection to the protein bound carbohydrates of the tissues.
Table 3.12: 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>3.26 ± 0.29</td>
<td>2.59 ± 0.21a*</td>
<td>3.29 ± 0.36</td>
<td>3.31 ± 0.33d'</td>
</tr>
<tr>
<td>Hexosamine</td>
<td>0.53 ± 0.05</td>
<td>0.38 ± 0.03a*</td>
<td>0.56 ± 0.04</td>
<td>0.54 ± 0.06d'</td>
</tr>
<tr>
<td>Hexuronic acid</td>
<td>0.30 ± 0.03</td>
<td>0.22 ± 0.02a*</td>
<td>0.32 ± 0.03</td>
<td>0.34 ± 0.04d'</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>28.00 ± 2.82</td>
<td>24.00 ± 2.26a*</td>
<td>28.23 ± 2.52</td>
<td>28.39 ± 2.55d'</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

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

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.
3.3.14 Tissue lipids

ADR, during chronic exposure, showed impaired lipid metabolism (Table 3.13) in the kidney. Increased cholesterol (both free and ester) and TG level, with a concurrent decrease in the PL and FFA levels were evidenced in the kidney of ADR administered rats (Group II). LA pretreatment (Group IV) significantly reduced cholesterol and TG levels (p<0.05), with a synergistic increase (p<0.05) in the levels of PL and FFA.

Endogenous abnormalities has been recently investigated to be involved in lipid metabolism causing glomerular damage (Grond et al., 1984). The importance of defining the precise role of lipid abnormalities in the pathogenesis of glomerulosclerosis is underscored by the almost invariable presence of hyperlipidemia in patients with renal disease.

Nephrotic syndrome was the result of a systemic abnormality in lipid metabolism for which the term 'lipoid nephrosis' had been coined (Munk, 1916). It has been reported that lipid deposits were not directly produced by the kidney, but resulted from the renal infiltration of plasma lipids that were frequently elevated in patients with the nephrotic syndrome.

ADR induces a severe nephrotic syndrome with massive proteinuria hyperlipidemia and hypoproteinemia (Montilla et al., 1997; Montilla et al., 1998). A significant hike in cholesterol levels of both the FC and to a greater extent EC fractions was evident from the present results. The present findings are in agreement with that of Badary et al. (2000) who has reported an increase in the TG and TC contents in the kidney of ADR administered rats.
Table 3.13: Effect of adriamycin (ADR) and lipoic acid (LA) on renal lipid fractions

<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>TC</td>
<td>5.35 ± 0.59</td>
<td>7.11 ± 0.64a*</td>
<td>5.16 ± 0.62</td>
<td>5.28 ± 0.48d*</td>
</tr>
<tr>
<td>FC</td>
<td>3.83 ± 0.30</td>
<td>4.36 ± 0.36a*</td>
<td>3.78 ± 0.34</td>
<td>3.80 ± 0.38d*</td>
</tr>
<tr>
<td>EC</td>
<td>1.52 ± 0.12</td>
<td>2.75 ± 0.25a*</td>
<td>1.38 ± 0.14</td>
<td>1.48 ± 0.13d*</td>
</tr>
<tr>
<td>TG</td>
<td>7.09 ± 0.70</td>
<td>8.98 ± 0.72a*</td>
<td>7.19 ± 0.65</td>
<td>7.13 ± 0.71d*</td>
</tr>
<tr>
<td>PL</td>
<td>14.93 ± 1.19</td>
<td>10.34 ± 0.72a*</td>
<td>15.11 ± 1.81</td>
<td>15.03 ± 1.20d*</td>
</tr>
<tr>
<td>FFA</td>
<td>3.02 ± 0.24</td>
<td>2.59 ± 0.23a*</td>
<td>3.12 ± 0.25</td>
<td>2.98 ± 0.36d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

mg per g wet tissue.

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.
Venketasan et al. (1991) have also reported an increase in the cholesterol content in the serum of ADR administered rats. Manabe et al. (2001) reported hypercholesterolemia in rats administered ADR. ADR administration may lower the level of cytochrome P450 which may in turn depress cholesterol 7α-hydroxylase activity, the key enzyme in the conversion of bile acids. Thus the present results corroborate the effectiveness of ADR as inducer of hyperlipidemic nephropathies similar to those described by other researchers.

Phospholipids are essential structural components of animal cell membranes and cytoskeletons (Stryer). The decrease in tissue phospholipid may perhaps be due to the increased activity of phospholipase, could be attributed to a marked decrease in phosphatidyl ethanolamine in basolateral membranes of the proximal tubules. A decline (p<0.05) in the FFA content of renal tissue was also observed in the present study.

It is well recognised that DHLA accumulates in the lipid portion of the membrane (Pagani et al., 1989). Kaiser et al. (1989) reviewed that LA inhibits the synthesis of cholesterol and its esterification by rat liver microsomes. Studies by Wagh et al. (1987) in diabetic rats displayed that LA reduces the formation of acetoacetyl DTO and HMG DTO, precursors for biosynthesis for cholesterol. Thus LA brings down cholesterogenesis. Furthermore they showed that in pharmacological concentrations, LA was capable of substituting -CoA in important steps of fat metabolism (enhancement of phospholipid biosynthesis without concomitant activation of cholesterol formation).

LA reduced tissue cholesterol and raised PL level in calcium oxalate stone forming rats (Jayanthi and Varalakshmi, 1992). Hence LA administration
to experimental rats, affords protection to membrane phospholipids and simultaneously exhibits anticholesterol action as brought out by the present results.

3.3.15 Serum lipids and lipoproteins

Hyperlipidemia is often present in patients with the nephrotic syndrome (Short et al., 1986). TC levels may be increased tenfold. Hypertriglyceridemia may also be present, but is usually less severe. The lipoprotein patterns associated with the nephrotic syndrome are quite variable. LDH and/or VLDL levels are often elevated (New Mark et al., 1975) whether HDL levels are also altered is controversial. Indeed HDL has been reported to be decreased, normal or even elevated (Sasaka et al., 1985).

The lipid composition of lipoprotein fractions may be abnormal in nephrotic syndrome. VLDL may contain an unusually high cholesterol concentration (Newmark et al., 1975). In general, the degree of lipoprotein abnormality is dependant on the magnitude of the proteinuria (Appel et al., 1985). Increased lipoprotein synthesis, therefore, probably plays an important role in hyperlipemia of the nephrotic syndrome (Marsh and Sparks, 1979). However, there is also evidence that decreased lipoprotein catabolism may cause elevations in plasma lipids in the nephrotic syndrome (Goldberg et al., 1977).

The serum lipid profile is generally considered as a reflection of the tissue metabolism and the permeability of cell membrane to various ions depends on lipid composition. Changes in serum lipid profile and lipoproteins are presented in table 3.14.
Table 3.14: Effect of adriamycin (ADR) and lipoic acid (LA) on serum lipids and lipid fractions

<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>TC</td>
<td>76.77 ± 8.45</td>
<td>120.36 ± 14.4a</td>
<td>74.86 ± 8.92</td>
<td>78.02 ± 8.60d*</td>
</tr>
<tr>
<td>FC</td>
<td>28.21 ± 2.53</td>
<td>38.32 ± 2.86a</td>
<td>27.33 ± 2.28</td>
<td>27.19 ± 2.36d*</td>
</tr>
<tr>
<td>EC</td>
<td>48.56 ± 4.32</td>
<td>82.04 ± 6.60a</td>
<td>47.53 ± 3.79</td>
<td>50.83 ± 4.41d*</td>
</tr>
<tr>
<td>TG</td>
<td>70.03 ± 6.03</td>
<td>80.33 ± 9.09a</td>
<td>69.89 ± 6.89</td>
<td>69.36 ± 7.63d*</td>
</tr>
<tr>
<td>PL</td>
<td>93.89 ± 9.34</td>
<td>120.97 ± 9.19a</td>
<td>95.36 ± 8.60</td>
<td>102.35 ± 10.20d*</td>
</tr>
<tr>
<td>FFA</td>
<td>38.36 ± 3.06</td>
<td>46.39 ± 3.99a</td>
<td>36.38 ± 2.87</td>
<td>40.71 ± 3.66d*</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>23.36 ± 2.16</td>
<td>48.24 ± 4.53a</td>
<td>23.11 ± 1.82</td>
<td>25.92 ± 2.16d*</td>
</tr>
<tr>
<td>VLDL-Cholesterol</td>
<td>10.24 ± 0.96</td>
<td>24.16 ± 2.00a</td>
<td>10.71 ± 0.95</td>
<td>11.36 ± 1.16d*</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>38.75 ± 3.23</td>
<td>50.36 ± 4.37a</td>
<td>39.26 ± 3.76</td>
<td>41.74 ± 3.34d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

mg per dl.

Comparisons were made between "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.
Serum TC and TG was significantly elevated in group II rats (p<0.05). A similar increase in serum cholesterol and TG has earlier been observed in ADR intoxicated rats (Montilla et al., 1997; Paczek et al., 1992). The increase may be related to reduction in the rate of 7α hydroxylation due to ADR. This metabolic effect may induce hypercholesterolemia. An increase in plasma cholesterol is the major lipid abnormality in many kidney diseases (Kane, 1994).

An increase in the content of PL in serum of ADR administered rats observed by Montilla et al. (1997), is consistent with our result. This study virtually indicates the preferential reactivity of ADR towards membrane PL (Blum and Carter, 1974) which is critical for the maintenance of mitochondrial function.

The decrease in the tissue PL may perhaps be due to the increased activity of phospholipase which eventually releases large amount of fatty acids in the serum of ADR administered rats. The decrease in tissue PL observed in the present results correlate with that of Iliskovic et al. (1998) and Shinya et al. (1997).

In the present study, LA was found to result in reduced (p<0.05) serum and tissue cholesterol levels. LA is reported to substantially reduce lipemia in cholesterol fed rats (Angeluci and Mascitelli-Coriandoli, 1958). Lukomskii (1964) reported that 75 mg of LA administered daily to cholesterol-fed rabbits reduced the cholesterol to PL ratio (LDL/HDL) in their plasma. LA exhibits cholesterol lowering action as evident from the reduced level noticed.

Lipoproteins in plasma exist as an emulsion by associating with non-polar lipids, amphipathic lipids and proteins, to make water miscible compounds.
LA promotes oxidation of FFA, thereby reducing their levels. Plasma FFA serves as a chief precursor for the fatty acid moiety of TG and the rate of hepatic TG synthesis and secretion (as VLDL) is a direct function of plasma FFA levels typically reducing LDL cholesterol (Barter et al., 1972).

It can be seen from the results presented that ADR administered to rats caused a significant (p<0.05) increase in LDL-cholesterol, VLDL-cholesterol and a marginal elevation in HDL-cholesterol levels as has been reported earlier by Hong et al., 2002; Calandra et al., 1983 and Okasora et al., 1992 respectively. Our results are conformable with the above reports. The increased level of VLDL and LDL might be due to over production of lipoproteins induced by hypoalbuminemia which is associated with ADR nephrotoxicity (Montilla et al., 1997).

3.3.16 Lipid metabolising enzymes

The activities of lipid metabolising enzymes of the kidney is depicted in table 3.15. A significant (p<0.05) decline in LPL activity with a concomitant increase in the activity of CES was observed in rats administered with ADR. The activities of these enzymes were restored to near normal levels upon pretreatment with LA (Group IV). No significant changes in the activity of CEH was noted in both the control and treated groups.

Thayer (1985) had reported a similar observation with reference to the activity of LPL in ADR administered animals. LPL activity varies inversely with FFA levels (Mc Carty, 1982). Decrease in plasma FFA caused increased LPL activity with LA treatment. LA promotes FFA oxidation which reduces the FFA levels.
Table 3.15: Effect of Adriamycin (ADR) and Lipoic acid (LA) administrations on lipid metabolizing enzymes in the 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>CES</td>
<td>13.69 ± 1.23</td>
<td>15.72 ± 1.61a*</td>
<td>13.16 ± 1.58</td>
<td>13.39 ± 1.34d†</td>
</tr>
<tr>
<td>CEH</td>
<td>14.21 ± 1.27</td>
<td>14.28 ± 1.14</td>
<td>13.92 ± 1.53</td>
<td>14.37 ± 1.48</td>
</tr>
<tr>
<td>LPL</td>
<td>14.96 ± 1.94</td>
<td>11.63 ± 1.64a*</td>
<td>14.08 ± 1.68</td>
<td>14.44 ± 1.29d†</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in table 3.1.

One unit of enzyme activity is expressed as: CES and CEH - one nmole of cholesterol; LPL - μmoles of free fatty acid liberated per mg protein per hour.

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

Values are statistically significant at *p < 0.05.
Norman, 1978 proposed that LPL activity is an important factor regulating HDL-cholesterol levels. The presence of LA caused increased LPL, which plays a key role in the reduction of serum LDL-cholesterol. The ability of LA in ameliorating ADR induced hyperlipidemic nephropathy in rats has thus been highlighted.

### 3.3.17 Serum constituents

Table 3.16 depicts the effects of ADR and LA administrations on certain serum constituents. ADR intoxication caused a significant increase (p<0.05) in serum urea, uric acid and creatinine. On comparison with group II animals pretreatment with LA (Group IV) was effective in preventing elevation in the levels of these serum constituents.

Nephropathy may be expressed as a decrease in GFR leading to reduced renal clearance of creatinine and uric acid and this leads to increased concentration of these substances in blood (Renkin and Robinson, 1974). A significant (p<0.05) increase in serum urea, uric acid and creatinine was observed in rats administered with ADR (Group II), which is in consonance with the results of Badary et al., 2000, Ghiggeri et al., 1990 and Okasora et al., 1992 respectively.

### 3.3.18 Urinary constituents

Table 3.17 shows the effects of ADR and LA on certain urinary constituents. A significant increase (p<0.05) was observed in the excretion of urea, uric acid, creatinine, phosphorous, protein and GAG’s in the urine of ADR administered animals. Compared to the ADR administered group, pretreatment
Table 3.16: Effect of adriamycin (ADR) and lipoic acid (LA) on serum 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>26.83 ± 2.41</td>
<td>41.91 ± 4.19a*</td>
<td>25.89 ± 2.07</td>
<td>26.13 ± 2.87d*</td>
</tr>
<tr>
<td>Uric acid</td>
<td>4.68 ± 0.42</td>
<td>6.77 ± 0.58a*</td>
<td>4.70 ± 0.33</td>
<td>4.73 ± 0.43d*</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.49 ± 0.15</td>
<td>2.37 ± 0.21a*</td>
<td>1.51 ± 0.17</td>
<td>1.55 ± 0.12d*</td>
</tr>
</tbody>
</table>

urea, uric acid, creatinine - mg per dl.

Treatment of groups are as in table 3.1.

Values represent the mean ± standard deviation 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.
with LA significantly increased the excretion levels of these urinary constituents group IV animals.

ADR has a broad spectrum of effects on the kidney, producing significant alterations in almost all potential indicators of nephrotoxicity measured in the urine. Urinary excretions of nitrogenous waste products (urea, uric acid and creatinine) was increased significantly in ADR injected rats coupled with phosphaturia. Elevation of the urinary urea might be a consequence of impaired solute transport in the proximal tubules. Rejection of solutes in the proximal tubule would retard water reabsorption and ultimately enhance fluid delivery to the distal nephron. This would increase the driving force for K+ secretion in the distal tubule and decrease the driving force for urea reabsorption in the collecting duct (Valtin, 1983). Ghiggeri et al. (1990) reported an increase in uric acid elimination in rats treated with ADR.

Proteinuria is a sensitive indicator for renal damage (Raab, 1972). ADR treated rats developed heavy proteinuria, which indicates extensive renal damage. This is due to the direct local toxic effect of ADR on the kidney (Bertani et al., 1982).

ADR has been demonstrated to cause increased urinary excretion of GAG's and loss of glomerular GAG's in rats (Benjelloun et al., 1993; Venkatesan et al., 2000). The observation that LA pretreatment was accompanied by significant reduction in the urinary GAG's in ADR administered rats suggests that this treatment may influence the integrity of glomerular basement membrane since the basement membrane is subjected to damage in ADR nephrosis (Raats et al., 1997). However, it cannot be excluded that the urinary excretion of GAG's
Table 3.17: 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>16.07 ± 1.45</td>
<td>18.26 ± 1.46a*</td>
<td>16.12 ± 1.45</td>
<td>15.96 ± 1.75d*</td>
</tr>
<tr>
<td>Uric acid</td>
<td>6.26 ± 0.69</td>
<td>7.44 ± 0.60a*</td>
<td>6.28 ± 0.50</td>
<td>6.30 ± 0.57d*</td>
</tr>
<tr>
<td>Creatinine</td>
<td>9.84 ± 0.78</td>
<td>17.46 ± 1.57a*</td>
<td>9.14 ± 0.73</td>
<td>10.13 ± 0.71d*</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.78 ± 0.07</td>
<td>2.85 ± 0.26a*</td>
<td>0.77 ± 0.06</td>
<td>0.82 ± 0.06d*</td>
</tr>
<tr>
<td>Protein</td>
<td>16.28 ± 1.79</td>
<td>36.26 ± 4.35a*</td>
<td>16.20 ± 1.46</td>
<td>16.33 ± 1.86d*</td>
</tr>
<tr>
<td>GAG's</td>
<td>12.76 ± 1.65</td>
<td>16.38 ± 1.97a*</td>
<td>12.73 ± 1.78</td>
<td>12.98 ± 1.16d*</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation for six rats.

Treatment of groups are as in Table 3.1.

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.
can be a consequence of proteinuria (Benjelloun et al., 1993). The phosphate excretion in the urine was also increased indicating that ADR exerts the main effect on the proximal tubules. Thus LA serves as an effective antidote in reducing tubular damage brought about by ADR administration.

3.3.19 Urinary enzymes

Toxic nephropathy is caused by several types of chemical compounds (Schreiner and Maher, 1965), whose effects, to a large degree, may be localised in proximal tubules. It was suggested that renal cellular damage might result in the release of cellular enzymes into urine (Rosalki and Wilkinson, 1959). A number of invasive and non-invasive methods have been developed over the past decade to detect deterioration of renal function in an early stage. One of the non-invasive tests is the measurement of urinary enzyme excretions.

The effect of ADR and LA administrations on certain urinary enzymes viz., ALP, ACP, LDH, LAP, β-glu, β-gal, NAG and γ-GT is depicted in figures 3.2a and 3.2b. ADR administered rats (group II animals) showed a significant (p<0.05) increase in the activities of these enzymes that were maintained at near normal levels on LA pretreatment (group IV animals).

When numerous tubular cells disintegrate or when tubular permeability is disturbed, renal enzyme excretion is markedly increased. Furthermore, new enzymatic activities deriving from kidney cells may appear in urine.

All disease states accompanied either by necrosis, decomposition or desquamation of renal tubular cells or by disturbances in glomerular filtration,
Fig 3.2a Effect of ADR and LA on certain urinary enzymes (ALP, ACP, LDH and LAP)

Bars represent the mean ± SD for six rats
ALP, ACP - μ moles of phenol, LDH - μ moles of pyruvate, LAP - μ 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
provoke increased ALP activity in urine: acute glomerulonephritis, renal infarction, acute tubular necrosis, glomerulosclerosis etc. The present investigation revealed an increase in the activity of ALP in rats administered with ADR. Moreover short periods of hypoxia (Dietz et al., 1967) provoke increased ALP activity through loss of brush borders and by inducing changes in tubular permeability. Urinary excretion of ACP, another lysosomal enzyme was found to be increased in ADR administered rats (Group II). This might be due to the degradation of lysosomes, leading to an increased urinary excretion of ACP.

The kidney is probably the major contributor to urinary LDH. Increased excretion of LDH, a cytoplasmic marker enzyme, can be considered as a biochemical marker of nephron cell damage. Hence, increased urinary LDH activity in the experimental rats is suggestive of tubular lesions. LDH determination is thus valuable in predicting early nephrotoxicity and proximal tubular damage. The observed increase in the excretion of LDH in the urine of animals administered with ADR may probably be indicative of proximal tubular damage due to the aminoglycoside.

Urinary β-Glu derives from the kidneys (lysosomes of tubular cells) and from the epithelial cells of the urinary tract (Raab, 1972). All acute inflammatory and toxic renal lesions are accompanied by an increased urinary β-Glu activity eg. tubular necrosis (Gonick and Schapiro, 1968). Increased excretion of β-Glu in urine is attributed to toxic kidney damage and similar results were seen in the present investigation of chronic ADR nephrotoxicity. Toxic damage to the renal tissues was also evident from increased (p<0.05) excretions of β-Gal.
Fig 3.2b Effect of ADR and LA on certain urinary enzymes (NAG, Y-GT, B-Glu and B-Gal)

Bars represent the mean ± SD for six rats

NAG - n moles of paranitrophenol; Y-GT - u moles of paranitroaniline; B-Glu, B-Gal - 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
NAG is one of the sensitive hydrolytic enzymes, which is released in urine after renal tubular damage (Whiting and Brown, 1996). The increase in NAG activity in urine could be a result of ADR induced damage to lysosomal membrane and a possible leak leading to its excretion in the urine. Thus NAG activity is detected in urine after toxic renal damage (Robinson et al., 1967). In this respect the present results are in par with that of Venkatesan et al. (2000) who observed a large increase in the urinary excretion of NAG.

γ-GT, a brush border enzyme is more deeply localized in the membrane. Determination of γ-GT proves useful in the diagnosis of proximal tubular damage. The present results corroborated with that of Palla et al. (1985) who has reported an increase in γ-GT after the administration of aminoglycosides. The increased excretion probably means damage to the proximal tubular epithelium and may reflect accelerated turn over of brush border membrane (Kaloyanides and Pastoriza-Munoz, 1980).

Toxic renal damage provokes a rise in urinary LAP activity. Increased urinary LAP activity was also observed in acute tubular necrosis (Scheler and Bergmann, 1964) caused by toxic substances. LAP activity is mainly present in proximal tubules and is capable of hydrolysing L-leucyl peptides. Substances with antifibrinolytic activity decrease urinary LAP activity. Since ADR possibly affects the proximal parts of the tubuli, which have been demonstrated to contain high activities of LAP, an increase in LAP activity was observed in the urine of ADR administered rats. Mohandass et al. (1995) have reported that lipoate effectively prevented the urinary biomolecular loss in gentamicin treated rats. Pretreatment
Histopathological changes in the kidney upon chronic administration with adriamycin.

Plate 3.1a: Group I - Section of kidney showing normal architecture (H & E x 100).

Plate 3.1b: Group II - Section of kidney showing an increase in glomerular cellular proliferation (H & E x 100).

Plate 3.1c: Group II - Section of kidney showing crescent formation (H & E x 100).

Plate 3.1d: Group III - Section of kidney showing focal hyalinisation leading to glomerulonephritis (H & E x 400).

Plate 3.1e: Group IV - Section of kidney showing normal morphology (H & E x 100).

Plate 3.1f: Group IV - Section of kidney showing almost normal architecture (H & E x 100).
with LA afforded protection against ADR-induced toxicity by minimising the renal enzyme leakage into the urine.

### 3.3.20 Morphological analysis

Histopathological studies of kidneys of the different groups of experimental rats revealed the following. The kidney of ADR administered animals showed an increase in glomerular cellular proliferation (3.3b), glomerular epithelial proliferation (3.3c) (crescent formation) and focal hyalinisation (33d). Examination of paraffin sections stained with hematoxylin-eosin failed to reveal consistent differences in the histology of LA pretreated ADR administered animals (3.3f) when compared to controls (3.3a). LA pretreated controls rats showed no significant changes (3.3e).