5. EVALUATION OF THE EFFECT OF LIPOIC ACID ADMINISTERED ALONG WITH GENTAMICIN, IN RATS RENDERED BACTEREMIC

5.1 INTRODUCTION

Gentamicin, a nephrotoxin is reported to be directly tubulotoxic (Rosen et al., 1994), and brings about many functional and biochemical derangements in the kidney (Bennet, 1983; Kaloyanides, 1984; Hottendorf and Williams, 1986; Looper and Bennet, 1987; Tulken, 1989). Reduced glomerular filtration rate, polyuria and renal phospholipidosis accompanied by enhanced urinary phospholipid excretion of enzymes and protein have been observed.

This antibiotic, inspite of its tubulotoxic effect, is most widely used for treating infections of *E.coli, Klebsiella, Proteus* and *Enterobacter*. On the other hand, DL $\alpha$-lipoic acid, a sulphhydryl compound has been reported to decrease gentamicin induced proteinuria and urinary phospholipid excretion (Mohandass et al., 1995). Hence this study was undertaken to evaluate the extent of nephrotoxicity and the degree of protection afforded by lipoate under *E.coli* infected conditions in rats and to note the effect of lipoic acid on the antimicrobial activity of gentamicin.
5.2 MATERIALS AND METHODS

5.2.1 Experimental animals

Male albino rats of the Wistar strain, weighing between 160 ± 20g obtained from King Institute of Preventive Medicine, Madras were used for this study. Animals were acclimatised to the animal house conditions for a week. Standard pellet feed (Brook Bond Lipton India Ltd., India) and clean drinking water was given ad libitum.

5.2.2 Fine chemicals

Fine chemicals for the experimental work were procured as indicated in section 2.2.2.

5.2.3 Experimental setup

Rats were divided into four groups of six animals each. Treatment to various groups were given as mentioned below. The duration of treatment was for 10 days in all the groups.

Group I : Animals served as controls.

Group II : Animals were injected intraperitoneally for 2 successive days with 0.2 ml inoculum containing $10^{10}$ colony forming units of *Escherichia coli* (clinical isolate, tested for gentamicin sensitivity, obtained from the Department of Microbiology, Dr.ALM PGIBMS, University of Madras, Chennai - 113).
Group III Animals were injected *E.coli* as those in Group II, in addition gentamicin 100 mg/kg was administered intraperitoneally for 10 successive days.

Group IV Animals received intraperitoneal injections of *E. coli* as above, plus gentamicin and also received lipoic acid (25 mg/kg) for 10 days by oral gavage.

Urine cultures for *E.coli* were obtained 48 hr after the second *E.coli* injection.

At the end of the experimental period, 24 hr urine samples were collected in cold containers using metabolic cages. The animals were killed by decapitation and blood was collected. Kidney and liver tissues were excised immediately and washed in ice-cold saline. A portion of the tissues were removed and fixed in 10% formalin solution for histological analysis. The remaining portion was then homogenised in ice-cold 0.1 M Tris-HCl buffer (pH 7.4) to give a 10% homogenate which was then used for biochemical studies.

5.2.4 Biochemical investigations

Biochemical analyses were made in urine, plasma, liver and kidney tissues.

5.2.4.1 Urine analyses

The following constituents were analysed in the urine.
a) **Urea**

Urine urea was estimated by the method of Natelson *et al.* (1951) by measuring the coloured complex formed with diacetylmonoxime in acidic medium as in section 2.2.6.1.

The values were expressed as mg/24 hr.

b) **Uric acid**

Uric acid was estimated by the method of Caraway (1963) as in section 2.2.6.2.

The values were expressed as mg/24 hr.

c) **Creatinine**

Creatinine content of urine was estimated by the method of Owen *et al.* (1954) as in section 2.2.7.1.

The values were expressed as mg/24 hr.

d) **Protein**

Protein was estimated by the method of Lowry *et al.* (1951) as in section 2.2.6.4.

The values were expressed as mg/24 hr.

e) **Urinary enzymes** like β-N-acetyl-D-glucosaminidase (NAG), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase,
(γ-GT) and cathepsin-D were assayed as indicated in sections 2.2.6.5.1, 2.2.6.5.4, 2.2.6.5.3., 2.2.6.5.2 and 2.2.8.3, respectively.

5.2.4.2 Analyses in Plasma

1 ml of plasma was diluted with 7 ml of distilled water and 1 ml of sodium tungstate and 1 ml of 2/3N sulphuric acid were added and centrifuged. Different aliquots of the supernatant were taken for the estimation of plasma urea, uric acid and creatinine.

a) Urea

Plasma urea was estimated by the method of Natelson et al. (1951) as in section 2.2.6.1.

Values were expressed as mg/dl.

b) Uric acid

Plasma uric acid was estimated by the method of Caraway (1963) as in section 2.2.6.2.

Values were expressed as mg/dl.

c) Creatinine

Creatinine level in plasma was estimated by the method of Owen (1954) as in section 2.2.7.1.

Values were expressed as mg/dl.
d) Creatinine clearance (Ccr)

Ccr was arrived at using a standard formula.

\[
\text{Ccr (ml/min)} = \frac{\text{Urine creatinine (mg/dl)} \times \text{Urine volume (ml/min)}}{\text{Plasma creatinine (mg/dl)}}
\]

5.2.4.3 Analyses in liver

Liver transaminases-aspartate transaminase (AST) and alanine transaminase (ALT) and lactate dehydrogenase (LDH) were assayed as mentioned in sections 2.2.8.1, 2.2.8.2, and 2.2.6.5.3.

5.2.4.4 Analyses in kidney

Alkaline phosphatase (ALP), cathepsin-D and lactate dehydrogenase (LDH) were assayed as mentioned in sections 2.2.6.5.4, 2.2.8.3 and 2.2.6.5.3, respectively.

Acid phosphatase (EC 3.1.3.2; ACP) activity in the kidney was assayed by the method of King (1965b) as mentioned below.

Reagents

1. Citrate buffer : 0.1 M pH 4.8.
2. Disodium phenyl phosphate : 0.1 M.
3. Sodium carbonate : 15% (w/v).
4. Folin - Ciocalteau reagent : Diluted 1:2 with water before use.
5. Standard phenol solution : 10 mg of phenol was dissolved in 100 ml of distilled water.

**Procedure**

The incubation mixture containing 1.5 ml of buffer and 1.5 ml of substrate was preincubated at 37°C for 10 min. Then 0.1 ml of enzyme preparation was added and incubated at 37°C for 30 min. After incubation, the reaction was arrested by the addition of 0.1 ml of Folin's phenol reagent. Enzyme was added to the control tubes after the addition of Folin's reagent. Then 1.0 ml of sodium carbonate (15%) was added to all the tubes. After 10 min, the blue colour developed was read at 640 nm.

The enzyme activity was expressed as μmoles of phenol liberated/mg creatinine/hr.

Renal lipid peroxidation, antioxidants like glutathione (GSH) and ascorbate and antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were evaluated as mentioned in sections 3.2.5, 3.2.6.1, 3.2.6.4, 3.2.7.2, 3.2.7.1 and 3.2.7.3.

Renal tissue ATPases namely Na⁺,K⁺-ATPase, Mg²⁺-ATPase and Ca²⁺-ATPase were also assayed as mentioned in sections 4.2.4.2.1, 4.2.4.2.3 and 4.2.4.2.2.
5.3 RESULTS AND DISCUSSION

The presence of *E. coli* in urine was determined 48 hr after the second injection of 0.2 ml inoculum containing $10^{10}$ colony forming units of *E. coli*. Group II animals revealed *E. coli* positive urine culture as shown in Table 5.1 and 50% of them died of *E. coli* poisoning. In group III, all animals survived the study period even though a few animals (34%) showed *E. coli* positive urine culture. *E. coli* positive urine culture was also noted in Group IV animals, but all animals survived the experimental period. Group IV rats showed survival rate similar to those injected only with *E. coli* and gentamicin (group III). In both the groups (Group III and IV) rats demonstrating *E. coli* positive urine cultures survived the study period. Thus the antimicrobial activity of gentamicin against *E. coli* in rats has not been affected in the presence of lipoic acid.

5.3.1 Constituents of urine and plasma

Table 5.2 shows the effect of gentamicin and lipoic acid administrations on urine and plasma urea, uric acid, urinary protein and creatinine clearance.

Proteinuria was detected in *E. coli* and *E. coli* plus gentamicin treated animals. The level of urea in the urine as decreased in both the groups. Proteinuria was more pronounced in group III animals when compared to group II animals.
Table 5.1: Effect of simultaneous administration of lipoic acid and gentamicin on the survival rate of rats rendered bacteremic

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Urine culture</th>
<th></th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Survival (%)</td>
<td>Mortality (%)</td>
</tr>
<tr>
<td>Group I</td>
<td>- (100%)</td>
<td>100</td>
<td>Nil</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>+ (100%)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>(E.coli)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>- (66%)</td>
<td>100</td>
<td>Nil</td>
</tr>
<tr>
<td>(E.coli + GM)</td>
<td>+ (34%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>- (66%)</td>
<td>100</td>
<td>Nil</td>
</tr>
<tr>
<td>(E.coli + GM + LA)</td>
<td>+ (34%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E.coli positive urine cultures are represented by ‘+’ and negative by ‘-’. Numbers given in the parenthesis represent the percentage of animals which showed either E.coli positive or negative urine culture.
Table 5.2: Effect of gentamicin and lipoic acid administrations on urinary and plasma constituents

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (E.coli)</th>
<th>Group III (E.coli + GM)</th>
<th>Group IV (E.coli + GM + LA)</th>
<th>F-ratio sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (mg/24 hr)</td>
<td>14.50 ± 0.98</td>
<td>27.51 ± 1.53a***</td>
<td>38.29 ± 1.79a<em><strong>b</strong></em></td>
<td>17.35 ± 1.27a<em><strong>b</strong></em>c***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Urea (mg/24 hr)</td>
<td>18.08 ± 0.89</td>
<td>13.48 ± 1.07a***</td>
<td>10.74 ± 0.77a<em><strong>b</strong></em></td>
<td>16.76 ± 1.42b<em><strong>c</strong></em></td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Uric acid (mg 24 hr)</td>
<td>6.61 ± 0.44</td>
<td>10.32 ± 0.72a***</td>
<td>6.96 ± 0.51b***</td>
<td>6.52 ± 0.34b***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>22.80 ± 1.95</td>
<td>19.80 ± 2.01</td>
<td>47.60 ± 2.97a<em><strong>b</strong></em></td>
<td>21.72 ± 1.08c***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.29 ± 0.21</td>
<td>3.10 ± 0.19</td>
<td>2.98 ± 0.27</td>
<td>3.31 ± 0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.97 ± 0.08</td>
<td>2.54 ± 0.11a***</td>
<td>3.14 ± 0.19a<em><strong>b</strong></em></td>
<td>2.24 ± 0.15a<em><strong>b</strong></em>c***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>1.02 ± 0.05</td>
<td>0.63 ± 0.02a***</td>
<td>0.37 ± 0.01a<em><strong>b</strong></em></td>
<td>0.98 ± 0.07b<em><strong>c</strong></em></td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.

Groups were treated as follows: Group I: Control; Group II - *E.coli* injected (0.2 ml inoculum containing 10^{10} colony forming units for 2 days successively by i.p.); Group III: *E.coli* injected as above + Gentamicin (100 mg/kg for 10 days, i.p.); Group IV - *E.coli* + Gentamicin + lipoic acid (25 mg/kg, for 10 days, orally).

Comparisons were made as follows: a - with group I; b - with group II; c - with group III.

*p < 0.05; **p < 0.01; ***p < 0.001; NS - Not significant.
The plasma level of urea was increased to a significant extent in group III.

The level of creatinine in the plasma was increased to a significant extent in both group II and group III animals. A concomitant fall in creatinine clearance was also observed in both the groups with a marked deterioration (0.37 ± 0.01 ml/min) in group III when compared with group II (0.63 ± 0.02 ml/min) animals.

These findings are consistent with those reported by Zager et al. (1986) and Smetana et al. (1992), namely that E.coli bacteremia and gentamicin exert synergistic nephrotoxicities, whereas E.coli without gentamicin-induced nephrotoxicity with mild azotemia.

Lipoic acid administration however, exhibited an antiproteinuric effect and increased the rate of creatinine clearance. This observation gains further support from the studies of Kalpana et al. (1994) where lipoic acid was reported to lower proteinuria and phosphaturia in mercuric chloride-induced acute renal dysfunction.

5.3.2 Urinary enzymes

**Figure 5** shows the effect of lipoic acid and gentamicin administrations on urinary excretion patterns of enzymes.

The activities of the urinary enzymes NAG, ALP, LDH and γ-GT were significantly elevated in E.coli plus gentamicin administered group whereas
FIG 5: EFFECT OF GENTAMICIN AND LIPOIC ACID ADMINISTRATIONS ON THE ACTIVITIES OF URINARY ENZYMES

Each Histogram and the vertical line above represents mean ± SD of 6 animals in each group. Comparisons: a - with control; b - with E.coli; c - E.coli + GM
*p < 0.05; **p < 0.01; ***p < 0.001
E.coli administered animals showed an elevation in all the enzymes except γ-GT. Cathepsin-D did not any change in its excretion pattern in any of the groups. The activities of NAG, LDH and ALP were more pronounced in group III animals suggesting gentamicin induced damage to the brush border membrane and necrosis due to its accumulation in the proximal tubules.

Upon treatment with lipoic acid, the activities of all these enzymes were found to be decreased in the urine, revealing that the thiol compound can act as a nephroprotectant.

5.3.3 Liver enzymes

Table 5.3 shows the effect of gentamicin and lipoic acid administrations on the liver aminotransferases and lactate dehydrogenase. A decrease in the activity of aspartate aminotransferase was observed in the E.coli administered group whereas the other two enzymes did not show any change in their activities in any of the experimental groups. From the above result it can be concluded that neither E.coli injection nor E.coli plus gentamicin administration brought about any hepatotoxic effects.

5.3.4 Kidney enzymes

Table 5.4 presents the effect of simultaneous administrations of gentamicin and lipoic acid on the activities of ALP, ACP, Cathepsin-D, LDH, γ-GT, and NAG in the kidney.
Table 5.3: Effect of gentamicin and lipoic acid administrations on the activities of liver ALT, AST and LDH

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (E.coli)</th>
<th>Group III (E.coli + GM)</th>
<th>Group IV (E.coli + GM + LA)</th>
<th>F-ratio sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>1.15 ± 0.11</td>
<td>1.04 ± 0.11</td>
<td>0.99 ± 0.12</td>
<td>1.14 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>AST</td>
<td>0.18 ± 0.008</td>
<td>0.12 ± 0.011a***</td>
<td>0.15 ± 0.013a<strong>b</strong>*</td>
<td>0.17 ± 0.014b**<em>c</em></td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>LDH</td>
<td>0.67 ± 0.04</td>
<td>0.65 ± 0.05</td>
<td>0.64 ± 0.03</td>
<td>0.65 ± 0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.
Groups were treated as in Table 5.2.
Comparisons were made as in Table 5.2.
ALT, AST and LDH - μmoles x 10^4 pyruvate liberated/min/mg protein.
*p < 0.05; **p < 0.01; ***p < 0.001; NS - Not significant.
Table 5.4: Effect of gentamicin and lipoic acid administrations on the activities of kidney enzymes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (E.coli)</th>
<th>Group III (E.coli + GM)</th>
<th>Group IV (E.coli + GM + LA)</th>
<th>F-ratio sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>0.58 ± 0.04</td>
<td>0.57 ± 0.05</td>
<td>0.43 ± 0.02a***, b***</td>
<td>0.60 ± 0.07c***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>ALP</td>
<td>2.10 ± 0.10</td>
<td>2.20 ± 0.15</td>
<td>1.07 ± 0.08a***, b***</td>
<td>2.16 ± 0.20c***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>ACP</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.03</td>
<td>0.20 ± 0.04</td>
<td>0.14 ± 0.03c*</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Cathepsin - D</td>
<td>0.19 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.20 ± 0.03</td>
<td>0.17 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>γ-GT</td>
<td>2.00 ± 0.17</td>
<td>2.03 ± 0.21</td>
<td>1.98 ± 0.20</td>
<td>2.12 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>NAG</td>
<td>0.52 ± 0.03</td>
<td>0.35 ± 0.04a***</td>
<td>0.27 ± 0.03a***, b**</td>
<td>0.48 ± 0.06b***, c***</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.

Groups were treated as in Table 5.2.

Comparisons were made as in Table 5.2.

LDH - μmoles of pyruvate liberated/min; ALP and ACP - μmoles x 10^1 phenol liberated/min; Cathepsin - D - μmoles of tyrosine liberated/hr; γ-GT - μmoles of p-nitroaniline liberated/min; NAG - μmoles x 10^2 of p-nitrophenol formed/min/mg protein.

*p < 0.05; **p < 0.01; ***p < 0.001; NS - Not significant.
Acid phosphatase, cathepsin-D, and γ-GT did not show any alterations in their activities in any of the treated groups.

The *E.coli* administered group showed a significant decrease in the activity of NAG alone (*p* < 0.001) whereas *E.coli* plus gentamicin administered animals showed a decrease in the activities of alkaline phosphatase (ALP) (*p* < 0.001) lactate dehydrogenase (LDH) (*p* < 0.001), and a marked decrease in N-acetyl glucosaminidase (NAG) activity (*p* < 0.001) when compared with that of their respective controls.

The decreased activities of these enzymes might be due to the tubulotoxic effect of gentamicin. Moreover, many compounds that affect the kidney are reported to be potent inhibitors of enzymes containing sulphydryl groups (Price, 1982).

Lipoic acid administration brought about a significant increase in the activities of both ALP (*p* < 0.001) and NAG (*p* < 0.001). It has been reported that lipoate can protect against cadmium induced membrane injury (Mueller, 1989), and thus the increase observed in the activities of NAG and ALP might be a manifestation of the cytoprotective action of lipoic acid.

### 5.3.5 Renal lipid peroxidation

*Table 5.5* shows the effect of lipoic acid and gentamicin administrations on **renal lipid peroxidation**.
Table 5.5: Effect of gentamicin and lipoic acid administrations on kidney lipid peroxidation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (E.coli)</th>
<th>Group III (E.coli + GM)</th>
<th>Group IV (E.coli + GM + LA)</th>
<th>F-ratio sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (LPO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.92 ± 0.06</td>
<td>1.27 ± 0.08***</td>
<td>1.62 ± 0.13a<em><strong>b</strong></em></td>
<td>1.09 ± 0.11a b c ***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Ascorbate-induced</td>
<td>4.98 ± 0.37</td>
<td>5.70 ± 0.54a***</td>
<td>6.83 ± 0.49a<em><strong>b</strong></em></td>
<td>5.02 ± 0.51b ***c ***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Ferrous sulphate-induced</td>
<td>7.52 ± 0.41</td>
<td>8.74 ± 0.52a***</td>
<td>9.50 ± 0.49a<em><strong>b</strong></em></td>
<td>7.62 ± 0.53b ***c ***</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.
Groups were treated as in Table 5.2.
Comparisons were made as in Table 5.2.
LPO - nmols of malondialdehyde formed/min/mg protein.
*p < 0.05; **p < 0.01; ***p < 0.001; NS - Not significant.
E. coli administration brought about a significant increase in renal lipid peroxidation and an added effect was observed when gentamicin was administered along with it.

Presence of a pathogen in the system activates phagocytosis, where, activated neutrophils produce superoxide, hydrogen peroxide and hypochlorous acid to kill the pathogen (Weiss, 1989; Babior and Woodman, 1990). Although the free radicals are produced only at the interface of the phagocyte plasma membrane and bacterium, some leakage of free radicals is inevitable. This is magnified when a large number of phagocytes becomes activated.

These free radicals can cause peroxidation of lipids and this might be the reason for the observed increase in lipid peroxidation in the E. coli treated group.

Gentamicin administration was reported to enhance the generation of hydrogen peroxide and superoxide anion which might interact to generate highly reactive and unstable oxidizing species, including the hydroxyl radical (Fantone and Ward, 1982; Weiss and DoBuglio, 1982; Fantone and Ward, 1985; Walker et al., 1987).

Hence the added effect observed in the case of E. coli plus gentamicin administered animals might be due to the action of free radicals liberated during phagocytosis and gentamicin-enhanced production.

Lipoic acid administration, however, decreased renal lipid peroxidation and this might be due to its direct free radical quenching ability
or due to its capacity to improve the status of the cellular antioxidants like glutathione.

5.3.6 Renal antioxidants

The status of enzymic and non-enzymic antioxidants in the kidney are depicted in Table 5.6.

In the E.coli administered group there was no change in the activities of SOD, GPx and in the levels of glutathione (GSH) and ascorbate.

In rats administered E.coli plus gentamicin a significant reduction in the activity of GPx, and the level of glutathione was observed. Catalase activity was also decreased in group III (p < 0.001).

The decrease in the activities of these enzymes might be due to increased oxidative stress placed on the cell as a result of gentamicin administration.

Lipoic acid treatment increased the glutathione status, subsequently improving the glutathione peroxidase activity as observed in group IV animals. It has been reported (Bast and Haenen, 1988) that lipoic acid can reduce oxidised glutathione and this may provide a rationale for the improved glutathione status in group IV animals.
Table 5.6: Effect of gentamicin and lipoic acid administrations on kidney antioxidants and antioxidising enzymes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (E.coli)</th>
<th>Group III (E.coli + GM)</th>
<th>Group IV (E.coli + GM + LA)</th>
<th>F-ratio sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>4.67 ± 0.39</td>
<td>4.69 ± 0.43</td>
<td>2.96 ± 0.27a <strong>b</strong></td>
<td>5.44 ± 0.42ab <strong>c</strong></td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>2.16 ± 0.11</td>
<td>2.18 ± 0.21</td>
<td>2.13 ± 0.17</td>
<td>2.90 ± 0.22ab <strong>c</strong></td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>CAT</td>
<td>198.25 ± 15.71</td>
<td>175.79 ± 20.05</td>
<td>131.46 ± 8.71ab <strong>c</strong></td>
<td>189.07 ± 10.05c **</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>SOD</td>
<td>3.25 ± 0.27</td>
<td>3.14 ± 0.41</td>
<td>3.33 ± 0.21</td>
<td>3.26 ± 0.24</td>
<td>NS</td>
</tr>
<tr>
<td>GPx</td>
<td>15.99 ± 1.23</td>
<td>16.40 ± 0.41</td>
<td>8.23 ± 0.91a <strong>b</strong></td>
<td>13.97 ± 1.27ab <strong>c</strong></td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.
Groups were treated as in Table 5.2.
Comparisons were made as in Table 5.2.
GSH, Ascorbate - μg/mg protein; CAT - μmoles of H₂O₂ consumed/min; SOD - Units/min (1 unit = the amount of enzymes required to bring about 50% inhibition in the auto-oxidation of pyrogallol); GPx - μmoles of GSH consumed/min/mg protein.
*p < 0.05; **p < 0.01; ***p < 0.001; NS - Not significant.
Table 5.7: Effect of gentamicin and lipoic acid administrations on the activities of kidney ATPases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (E. coli)</th>
<th>Group III (E. coli + GM)</th>
<th>Group IV (E. coli + GM + LA)</th>
<th>F-ratio sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺,K⁺-ATPase</td>
<td>0.69 ± 0.04</td>
<td>0.37 ± 0.02 ***</td>
<td>0.42 ± 0.03 ***</td>
<td>0.66 ± 0.06b *** c ***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Ca²⁺-ATPase</td>
<td>0.41 ± 0.03</td>
<td>0.42 ± 0.05</td>
<td>0.21 ± 0.02a ***b ***</td>
<td>0.39 ± 0.02c ***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Mg²⁺-ATPase</td>
<td>0.53 ± 0.03</td>
<td>0.51 ± 0.05</td>
<td>0.31 ± 0.01a ***b ***</td>
<td>0.54 ± 0.04c ***</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.

Groups were treated as in Table 5.2.

Comparisons were made as in Table 5.2.

ATPases - µmoles of Pi liberated/min/mg protein.

*p < 0.05; **p < 0.01; ***p < 0.001; NS - Not significant.
5.3.7 Renal ATPases

Table 5.7 shows the effect of gentamicin and lipoic acid administrations on renal ATPases in rats rendered bacteremic.

E. coli injection did not bring about any change in the activities of Ca\(^{2+}\)- and Mg\(^{2+}\)- ATPases. However, a significant reduction in the activity of Na\(^{+}\),K\(^{+}\)- ATPase was observed.

In group III animals there was significant decrease in the activities of Ca\(^{2+}\)-, Mg\(^{2+}\)- and Na\(^{+}\),K\(^{+}\)- ATPases. This result agrees with our previous findings (Sandhya et al., 1995) of decreased activities of phosphohydrolases in the kidney.

Lipoic acid treatment brought about a significant increase in the activities of all the three enzymes thus helping to maintain the ionic equilibrium.

5.4 HISTOLOGY

Histological study of kidney section obtained from E. coli injected animal showed a significant oncotic change in the epithelium of the proximal tubular cells (Fig. 5.1b) when compared with that of the control (Fig. 5.1a). Analyses of sections from E. coli plus gentamicin administered, and E. coli plus gentamicin plus lipoic acid administered animals showed a normal morphology (Fig. 5.1c and Fig. 5.1d) as that of the control (Fig. 5.1a).
Kidney

Fig. 5.1a : Group I - Section of kidney showing normal morphology. 
(H & E x 100)

Fig. 5.1b : Group II - Section of kidney revealing oncotic changes in the epithelium of proximal tubular cells. 
(H & E x 100)

Fig. 5.1c : Group III - Section of kidney showing normal architecture. 
(H & E x 100)

Fig. 5.1d : Group IV - Section of kidney showing normal morphology. 
(H & E x 100)
Liver

Section of liver of control (Fig. 5.2a), *E.coli* injected (Fig. 5.2b), *E.coli* plus gentamicin injected (Fig. 5.2d) and *E.coli* plus gentamicin plus lipoic acid administered (Fig. 5.2c) animals showing normal architecture.

(H & E x 100)
Histological analyses of sections of liver obtained from groups II, III and IV animals showed a normal architecture in all the groups (Figs. 5.2b, Fig. 5.2c and Fig. 5.2d) when compared to that of the sections from control (Fig. 5.2a).