Oxidative stress in paracetamol-induced pathogenesis: (I) Renal damage

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The effect of administration of paracetamol (1 g/kg body wt) on oxidative damage to proteins and lipids in the kidney was studied at various time intervals in adult male Wistar rats. Indicators of oxidative stress, such as protein thiol, protein carbonyl content and lipid peroxide levels were assayed along with thiol-dependent enzyme activities, glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase. Paracetamol-induced renal damage after 4 hr of administration was evidenced by elevation in plasma creatinine levels and the presence of acute tubular necrosis on histological examination of the kidney. No significant change in any other parameters was observed, except for decreased glutathione level. An increase in lipid peroxide level was observed at 24 hr after treatment. The results suggest that oxidative stress may not play a causative role, but contribute to the pathogenesis of paracetamol-induced renal damage.

Keywords: lipid peroxidation, protein thiol, protein carbonyl, paracetamol, renal damage

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Paracetamol, a widely used analgesic-antipyretic drug, though considered a safe drug in overdose situations, produces hepatic necrosis and renal failure in humans as well as other experimental animals. Its accidental or suicidal ingestion may result in death due to renal failure. Oxidative stress is reported to play an important role in the pathogenesis of paracetamol-induced liver damage. However, the mechanism of paracetamol-induced renal damage is not clear, though some studies suggest that lipid peroxidation plays a role, while others maintain that it may not. Although lipid peroxidation is a commonly used measure of oxidative stress, indicators such as protein thiol, protein carbonyl content are also measured as the proteins regulate various metabolic pathways in the body and damage to proteins may result in cellular dysfunction and cell death. Protein carbonyls content has a major advantage over lipid peroxidation products, as they are formed early and are stable, as compared to others, such as glutathione disulphide and malondialdehyde.

In the present study, protein thiol, protein carbonyl content, lipid peroxide levels and the activities of important thiol-dependent enzymes, glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase were assayed in the kidneys of rats at various time intervals after treatment with a toxic dose (1 g/kg body wt) of paracetamol. The dose was chosen as the lower doses (350, 500 and 750 mg/kg body wt) did not induce any renal damage. Paracetamol at the dose of 1 g/kg body wt or above was used earlier by other workers. Plasma creatinine level was also assayed, as it is a good indicator of renal damage.

Materials and Methods

Animal treatment

Adult male Wistar rats (wt 150-200 g) were housed in galvanised iron cages and maintained on standard laboratory rat chow with free access to water in a thermostatically controlled room (28°C) under 12 hr dark/light cycle. The experimental animals were administered phenobarbitone intraperitoneally @ 80 mg/kg body wt for 3 days. Phenobarbitone was administered, as it may enhance the nephrotoxicity of paracetamol, since it is an inducer of cytochrome P that metabolizes paracetamol to its toxic free radicals.

Rats were administered paracetamol intraperitoneally @ 1 g/kg body wt in 5% dimethylsulfoxide (DMSO) on the 4th day. Diluted DMSO was used to dissolve paracetamol, because when paracetamol was administered as a supersaturated solution at 40°C in 0.9% saline solution, it recrystallized when drawn into the syringe and, it would not dissolve in basic solution (pH 11.3) at 25°C in 0.9% saline solution. Phenobarbitone control rats were administered phenobarbitone for 3 days, followed by 5% DMSO on the 4th day and water control rats were administered sterile water.

Rats were sacrificed at various time intervals — 4, 24 and 40 hr after administration of paracetamol /vehicle, after withdrawal of blood under light ether anaesthesia. The kidneys were immediately removed.
and used for biochemical assays and histological assessment. The time intervals were chosen, as the renal damage between 24 hr to 48 hr after paracetamol administration as reported earlier could not be observed in the present study. Moreover, the renal damage was maximum at 4 hr, as evidenced by the highest plasma creatinine level after the administration of paracetamol.

**Histological studies**

Slices of the kidney tissue were fixed in buffered formalin, processed and stained with hematoxylin-eosin stain.

**Biochemical assays**

A 10% w/v homogenate of the kidney was prepared using Potter-Elvehjem homogeniser in ice-cold 10 mM/L HEPES buffer, pH 7.4, containing 125 mM/L KCl, 5 mM/L disodium EDTA and protease inhibitors leupeptin (0.5 mg/L), pepstatin (0.7 mg/L), aprotinin (0.5 mg/L) and phenylmethylsulphonyl fluoride (40 mg/L) to inhibit protease activity. The homogenate was centrifuged at 10,000 g for 20 min at 2°C and the supernatant used for the assay of protein carbonyl

A 10% w/v homogenate of the kidney was prepared in ice-cold isotonic KCl (1.15%), centrifuged at 10,000 g for 20 min at 2°C and the supernatant used for the assay of glyceraldehyde-3-phosphate dehydrogenase.

Malondialdehyde level was assayed by thiobarbituric method, using a 10% w/v whole homogenate of kidney. Glutathione was determined according to the method of Ellman, using the kidney homogenized in 4.5 volumes of ice-cold 0.1 M phosphate buffer, pH 7.4.

Protein content in the supernatants was assayed using Folin’s reagent. Plasma creatinine was estimated based on its reaction with picric acid in alkaline medium.

The data represent mean ± S D. Means of 3 groups were compared by analysis of variance. A p value of ≤ 0.05 was considered statistically significant.

**Results and Discussion**

The results of administration of paracetamol on different parameters at various time intervals are given in Table 1. Paracetamol induced acute renal damage as early as 4 hr after its administration, as indicated by elevation in plasma creatinine levels and occurrence of tubular necrosis histologically (Figure not shown). Depletion of glutathione (20%) was observed; however, no significant alteration in the other parameters measured was observed. At 24 hr, glutathione levels decreased further (28%) and

### Table 1 — Renal protein carbonyl, protein thiol, lipid peroxide, glutathione levels and the activities of glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase and plasma creatinine levels at different time intervals after treatment with paracetamol in experimental and control rats

<table>
<thead>
<tr>
<th></th>
<th>Protein carbonyl (nm/mg protein)</th>
<th>Protein thiol (nm/mg protein)</th>
<th>Glutathione (μmol/g)</th>
<th>Lipid peroxide (nm/g wet wt)</th>
<th>Glutamine synthase (U/mg protein)</th>
<th>Malondialdehyde (nm/g wet wt)</th>
<th>Glyceraldehyde-3-Plasma creatinine (mg/dl)</th>
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</thead>
<tbody>
<tr>
<td><strong>After 4 hr</strong></td>
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<tr>
<td>Water control (n=4)</td>
<td>2.48 ± 0.74</td>
<td>96.2 ± 5.5</td>
<td>1.12 ± 0.09</td>
<td>187.0 ± 28.0</td>
<td>2.20 ± 0.27</td>
<td>0.16 ± 0.047</td>
<td>0.67 ± 0.08</td>
</tr>
<tr>
<td>Paracetamol/phenobarbitone (n=8)</td>
<td>2.13 ± 0.47</td>
<td>100.0 ± 6.4</td>
<td>1.02 ± 0.04</td>
<td>162.0 ± 34.0</td>
<td>2.20 ± 0.25</td>
<td>0.14 ± 0.045</td>
<td>0.74 ± 0.33</td>
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<tr>
<td><strong>After 24 hr</strong></td>
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<tr>
<td>Water control (n=4)</td>
<td>1.86 ± 0.29</td>
<td>110.0 ± 9.0</td>
<td>1.08 ± 0.09</td>
<td>143.0 ± 8.6</td>
<td>1.86 ± 0.46</td>
<td>0.13±0.042</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Phenobarbitone (n=6)</td>
<td>2.07 ± 0.22</td>
<td>107.0 ± 9.1</td>
<td>1.00 ± 0.06</td>
<td>166.0 ± 27.0</td>
<td>1.96 ± 0.50</td>
<td>0.11 ± 0.031</td>
<td>0.68 ± 0.12</td>
</tr>
<tr>
<td>Paracetamol/phenobarbitone (n=8)</td>
<td>2.4±0.52</td>
<td>107.0±9.96</td>
<td>0.72 ± 0.04*</td>
<td>207.0±37.0*</td>
<td>2.1±0.34</td>
<td>0.10±0.034</td>
<td>0.73±0.11</td>
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<td><strong>After 40 hr</strong></td>
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<tr>
<td>Water control (n=4)</td>
<td>2.17 ± 0.51</td>
<td>133.0 ± 6.7</td>
<td>1.18±0.04</td>
<td>137.0±36.5</td>
<td>1.64±0.24</td>
<td>0.14±0.020</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td>Phenobarbitone (n=6)</td>
<td>2.97 ± 1.2</td>
<td>132.0 ± 7.0</td>
<td>1.07±0.05</td>
<td>138.0±18.0</td>
<td>1.61±0.22</td>
<td>0.13±0.026</td>
<td>0.79±0.07</td>
</tr>
<tr>
<td>Paracetamol/phenobarbitone (n=8)</td>
<td>2.2±0.69</td>
<td>138.0±6.9</td>
<td>1.12±0.11</td>
<td>191.0±41.0</td>
<td>1.44±0.17</td>
<td>0.11±0.015</td>
<td>0.76±0.14</td>
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*p < 0.05, **p < 0.01 as compared with the controls
malondialdehyde increased by 45% in the paracetamol-treated rats, as compared to the control. Other parameters were not significantly altered and no renal damage was evident (normal histology and normal levels of plasma creatinine).

Earlier, it was proposed that the cytotoxic mechanism involves oxidation of paracetamol by microsomal mixed function oxidases to the reactive metabolite, N-acetyl-p-benzoquinoneimine in liver and kidney, which acts as a potent arylating agent by covalently binding to tissue nucleophiles. Tissue glutathione is thought to play a protective role in forming adducts with these toxic metabolites; once glutathione stores are depleted, the free radical can react with the cellular macromolecules, such as lipids and proteins. Oxidative damage to lipids results in enhanced lipid peroxidation, and to proteins, an increase in carbonyl content and decrease in thiol. In the present study, protein carbonyl content, protein thiol, and the activities of glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase were not significantly altered after the administration of toxic dose of paracetamol, suggesting that the oxidative damage to renal proteins may not occur in paracetamol intoxication.

Although, there was a decrease in glutathione level, no increase in malondialdehyde levels was observed 4 hr after treatment with paracetamol, when renal damage was evident. Glutathione level was further decreased and malondialdehyde level increased 24 hr after treatment, when there was no evidence of renal damage, suggesting that the increased malondialdehyde level may be a consequence of glutathione depletion. Similar results were reported by others. Although, a recent study suggests that lipid peroxidation plays a role in paracetamol-induced renal damage is evident (normal histology and normal parameters were not significantly altered and no renal damage was evident). Also, hepatically-derived glutathione conjugates may be involved in paracetamol-induced renal injury. A recent report suggests that nitric oxide may play an important role in paracetamol-induced renal damage in rat. Although, oxidative stress is reported to play a role in paracetamol-induced liver damage, as evidenced by elevation in lipid peroxide levels, depletion of glutathione, decrease in protein thiol and in the activities of thiol-dependent enzymes, the present study suggests that it may contribute to the pathogenesis of paracetamol-induced renal damage.

References
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