Biochemical and structural changes of the kidney in mice exposed to phenol

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Aim: To investigate the histology and function of the kidney in mice exposed to different concentrations of phenol through the gavage method for 10 consecutive days.

Materials and methods: Forty female BALB/c mice were selected and randomly divided into 1 control group and 3 experimental groups. The control group was administered distilled water through the gavage method, while the experimental groups received daily doses of 80, 180, and 320 mg/kg of phenol, respectively. After 10 days, blood samples were collected and the sera were analyzed biochemically. Renal tissue samples were also taken and histopathological changes of the kidneys were examined using optical and electron microscopes.

Results: No significant differences were found in the serum levels of sodium, potassium, chloride, bicarbonate, or total protein in all of the phenol-treated mice in comparison with the control group. The levels of creatinine and blood urea nitrogen and the activity of alkaline phosphatase in the sera of the phenol-treated mice showed a significant increase in comparison with the control group (P < 0.05). Histological changes including renal tubules necrosis, interstitial lymphoplasmacytic nephritis, and hyperemia were observed. The ultrastructural changes included reduction in the number and size of microvilli in the epithelial cells of the proximal convoluted tubules (PCTs), deformation and shrinkage of the nuclei, malformation of the mitochondria and folding of the cytoplasm in the epithelial cells of the PCTs, dilation of the urinary space in the renal corpuscles, and formation of endothelial electron-dense deposits in the basement membranes of the glomeruli.

Conclusion: Based on the findings, it is concluded that the observed significant increases of serum creatinine levels, blood urea nitrogen levels, alkaline phosphatase activity, and histopathological changes in the kidneys of phenol-treated mice indicate tissue injury. These findings suggest that treatment with different concentrations of phenol may cause nephrotoxicity in mice.

Key words: Phenol, biochemical, histology, mice, kidney

Introduction

Phenol (C₆H₅OH), a monosubstituted aromatic hydrocarbon, is used to synthesize phenolic resins and plastics. Due to its anesthetic and disinfectant properties, phenol is also widely used in pharmaceutical products such as ointments, ear and nose drops, sprays, and antiseptic lotions (1,2). The kidney is the main target organ of phenol toxicity (3), and its elimination is mainly the task of the kidney in human and laboratory animals (4-6). It has been

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reported that phenol metabolism is done extensively in the kidney (7). The detrimental renal effects attributed to phenol toxicity include tubular necrosis, protein cast formation, and papillary hemorrhage (8). To the best of our knowledge, there is not a comprehensive study on renal biochemistry and ultrastructural changes of the kidney due to phenol administration. The purpose of the present study was to investigate the effects of phenol administration on the biochemical and histological integrity of the kidney in BALB/c mice.

Materials and methods

Chemical

The phenol (C₆H₅OH) used in this study was provided from Biochem Chemical Co. (Tehran, Iran) and dissolved in distilled water as a phenol carrier. Solutions of this chemical material at concentrations of 80, 180, and 320 mg/kg were prepared to provide the appropriate doses for the experiments.

Animals

For this study, 40 eight-week-old female BALB/c mice at 30 g of initial body weight were purchased from the Razi Institute (Karaj, Iran). There were 4 or 5 mice housed per stainless-steel cage under conventional conditions (temperature of 22 ± 1 °C, relative humidity of 50 ± 10%, and natural light/dark photoperiod of 12:12); they had ad libitum access to drinking water and food. The mice were allowed to acclimatize to the laboratory environment at for least 6 days before commencement of testing. All of the procedures were carried out in accordance with institutional guidelines for animal care and use.

Experimental design

The experiment was carried out for 10 consecutive days and the animals were randomly divided into 1 control group and 3 experimental groups, each comprising 10 mice. The control group received only distilled water, whereas the experimental groups received phenol at daily concentrations of 80, 180, and 320 mg/kg, respectively. The concentrations were determined on the basis of a prior study. The concentrations and stabilities of the chemical were also confirmed.

Blood collection

At 24 h after administration of the last doses, the animals were anesthetized with chloroform vapor, quickly brought out of the jar, and sacrificed. The whole blood was collected into sterilized vials after direct cardiac puncture. Blood samples were allowed to clot at 4 °C and centrifuged at 5000 rpm for 10 min. After separation of the sera, they were put into sterile tubes for measurement of the biochemical parameters.

Biochemical analysis

Serum levels of sodium and potassium were measured in advance using flame photometry (Corning, flame photometer model 410, Corning, NY, USA), while levels of chloride and bicarbonate were measured by titration procedures. Serum samples were analyzed for total protein by the Biuret method, for creatinine (CRT) according to the Jaffe method, for blood urea nitrogen (BUN) by the modified urease-Berthelot method, and for alkaline phosphatase (ALP) activity by the enzymatic (International Federation of Clinical Chemistry and Laboratory Medicine) method, using a spectrophotometer (Shimadzu, model AA200, Tokyo, Japan) and commercial colorimetric kits obtained from Pars Azmun Co. (Tehran, Iran).

Histopathological assessment for renal injury

For optical microscopy, immersion of the left kidney was maintained overnight in 10% neutral buffered formalin for fixation. The kidneys were then sectioned at 5 μm with a microtome and stained with hematoxylin and eosin (H&E). The sections were photographed directly using a stereomicroscope at 400× high-power fields with a Microsoft system. The following criteria were used for registering the histological changes of the kidney: (+++), a dominant change in all of the animals; (++), a relatively common change in all of the animals; (+), a change in a few of the animals; and (+), a change in a few of the animals.

For electron microscopy, small pieces (2 mm) of the left kidney were fixed in 2.5% glutaraldehyde. The specimens were put in 0.1 M phosphate buffer (pH 7.3) at 4 °C for 4 h. After 2 washings (30 min each) with cold 0.2 M phosphate buffer, the tissues were postfixed in 1% osmium tetroxide at 4 °C for 4 h. The specimens were dehydrated in a gradual
series of ethanol and embedded in EPON 812. Ultrathin sections (600 Å) were cut employing an ultramicrotome (Ultracut, Reichert-Jung, Vienna, Austria), mounted onto copper grids, and stained with uranyl acetate and subsequently with lead citrate (9). The grids were examined using a Zeiss 902 electron microscope (Carl Zeiss AG, Jena, Germany). In each group, 4 samples of renal cortex from each animal were selected and the severities of the lesions were evaluated. According to the changes in the renal tubules, glomeruli, and interstitium, the lesions were classified as mild (+), moderate (++), intense (+++), and severe (++++)..

**Data analysis and statistics**

All of the results were expressed as means and the standard errors of the means. Analysis of variance (ANOVA) was used to test the overall significance of differences among the means. Tukey-Kramer's multiple comparison test was applied for post hoc comparison. Computations were performed using SPSS 11.5 for Windows. P < 0.05 was considered significant.

**Results**

**Effect of phenol administration on the biochemical parameters**

The serum levels of sodium, potassium, chloride, bicarbonate, and total protein were not significantly affected, but the CRT, BUN, and activity of ALP in the sera of phenol-treated mice showed a significant increase in comparison with the control group (P < 0.05) (Table 1).

**Histopathological findings**

In the mice that received 80 mg/kg phenol, the kidney showed hyperemia. This alteration, as well as moderate interstitial lymphoplasmacytic nephritis, was observed in the group receiving 180 mg/kg. At 320 mg/kg, severe interstitial lymphoplasmacytic nephritis and necrosis of the renal tubules were also observed (Table 2, Figures 1 and 2). Other parts of the kidney showed no pathological changes.

**Ultrastructural findings**

Changes including reduction in the number and size of the microvilli in the epithelial cells of the

### Table 1. Some serum biochemical parameters in control and treated mice with different concentrations of phenol through gavage for 10 days.

<table>
<thead>
<tr>
<th>Parameters/groups</th>
<th>Control</th>
<th>80 mg/kg phenol daily</th>
<th>180 mg/kg phenol daily</th>
<th>320 mg/kg phenol daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol/L)</td>
<td>148.50 ± 3.07a</td>
<td>151.25 ± 2.23a</td>
<td>155.44 ± 1.18a</td>
<td>159.62 ± 2.06a</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>7.10 ± 0.71a</td>
<td>6.86 ± 1.33a</td>
<td>6.46 ± 0.99a</td>
<td>7.59 ± 1.27a</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>100.50 ± 1.07a</td>
<td>98.25 ± 1.54a</td>
<td>103 ± 1.18a</td>
<td>96.62 ± 1.18a</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>17.10 ± 0.09a</td>
<td>19.46 ± 0.34a</td>
<td>13.46 ± 0.29a</td>
<td>14.46 ± 0.16a</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>4.07 ± 0.69a</td>
<td>4.15 ± 0.14a</td>
<td>4.05 ± 0.34a</td>
<td>4.11 ± 0.11a</td>
</tr>
<tr>
<td>CRT (mg/dL)</td>
<td>0.29 ± 0.02a</td>
<td>0.91 ± 0.015b</td>
<td>1.27 ± 0.071b</td>
<td>0.99 ± 0.021b</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>31.87 ± 2.64a</td>
<td>48.75 ± 4.55b</td>
<td>49.22 ± 2.74a</td>
<td>53.37 ± 3.48b</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>56.33 ± 6.68a</td>
<td>115.12 ± 7.12a</td>
<td>133.01 ± 3.55b</td>
<td>138.21 ± 5.32b</td>
</tr>
</tbody>
</table>

Different letters indicated significant differences at P < 0.05.

ALP: alkaline phosphatase, BUN: blood urea nitrogen, CRT: creatinine.
Table 2. Summarized histological changes in the kidney of mice exposed to different concentrations of phenol by the gavage method for 10 days in comparison to the control animals.

<table>
<thead>
<tr>
<th>Findings/groups</th>
<th>Control</th>
<th>80 mg/kg phenol daily</th>
<th>180 mg/kg phenol daily</th>
<th>320 mg/kg phenol daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis of renal tubules</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Interstitial lymphoplasmacytic nephritis</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Lesion described as (+++): a dominant change in all of the animals of each group; (++): a relatively common change in all of the animals of each group; (+): a change in all of the animals of each group; (+): a change in a few of the animals of each group.

Figure 1. a) Kidney transverse section of the control mice showing normal renal tissue. b) Kidney transverse sections of the mice treated with phenol at a daily concentration of 180 mg/kg for 10 continuous days. This section shows interstitial lymphoplasmacytic nephritis (arrows) and necrosis (double-headed arrows) in the epithelial cells of the renal tubules. c and d) Kidney transverse sections of the mice treated with phenol at a daily concentration of 320 mg/kg for 10 continuous days. These sections show severe interstitial lymphoplasmacytic nephritis (arrows) and necrosis of renal tubules (double-headed arrows). D: distal convoluted tubules, G: glomerulus, and P: PCTs; hematoxylin and eosin stain, 400×.
proximal convoluted tubules (PCTs), deformation and shrinkage of the nuclei, deformity in the shape of the mitochondria and folding of the cytoplasm of the epithelial cells of the PCTs, dilation in the urinary space of the renal corpuscles, and formation of endothelial electron-dense deposits (EEDs) in the basement membranes of the glomeruli were seen in the mice that received phenol (Table 3, Figures 3 and 4).

**Discussion**

Estimation of the renal excretion of the waste metabolites and histological changes in the kidney has provided useful information on the health status of the kidneys (10). CRT and BUN are waste products of protein metabolism that have to be excreted by the kidney. Therefore, it is found that increases of CRT and BUN in this study are indicators of the biochemical damage to the kidney (10).
Table 3. Ultrastructural features of renal lesions observed in mice exposed to different concentrations of phenol by the gavage method for 10 days in comparison with the control group.

<table>
<thead>
<tr>
<th>Renal lesions/groups</th>
<th>Control</th>
<th>80 mg/kg phenol daily</th>
<th>180 mg/kg phenol daily</th>
<th>320 mg/kg phenol daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in the number and size of the microvilli in the epithelial cells of the PCTs</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Deformation and shrinkage of nuclei, malformation of mitochondria, and folding of the cytoplasm in the epithelial cells of the PCTs</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Dilation of the urinary space of the renal corpuscles</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Formation of EEDs in the basement membrane of the glomerulus</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

EEDs: endothelial electron-dense deposits, PCT: proximal convoluted tubules.
Lesion described as (++++) severe, (+++) intense, (+++) moderate, (+) mild, and (-) no lesion.

Figure 3. a) Electron micrograph of a part of a kidney in the control mice showing part of the lining cells of the PCTs. In this micrograph, 3 nuclei (N) and an abundant long sliver of the microvilli (MV) on the surface are observed (4400×). b) Electron micrograph of a part of a kidney in the mice treated with phenol at a daily concentration of 180 mg/kg for 10 continuous days, showing part of the PCT epithelial cells. In this micrograph, 2 N and decreases in the number and size of the apical MV in the PCT epithelial cells are observed (5000×). c) Electron micrograph of a part of the PCT lining cells in the control mice. In this micrograph, the N and the mitochondria (M) have a normal round shape. The interstitial space (IS) is also small in size (3000×). d) Electron micrograph of a part of a kidney in the mice treated with phenol at a daily concentration of 320 mg/kg for 10 continuous days, showing parts of 2 PCT epithelial cells. In this micrograph, N is deformed and has shrunk, M is fusiform in shape, IS is large in size, and the cytoplasm of the PCT epithelial cells is folded (star) (3000×).
Electrolytes and the water excretion balance are regulated via the kidneys; thus, the plasma electrolyte levels also may provide important information on the biochemical status of the kidney performance (11). Serum concentrations of electrolytes and excretory materials are important measures in assessing the biochemical capabilities of the kidney because they demonstrate the presence or absence of active lesions in the kidney and the biochemical capacity of the different parts of the nephron (10). Creatinine, urea, and electrolytes (sodium, potassium, chloride, and bicarbonate) are the most sensitive biochemical markers employed in the diagnosis of renal damage, since CRT and BUN are excreted through the kidney while the electrolytes are reabsorbed and excreted in the tubules (10).

Serum ALP activity is an indication of the possibility of kidney malfunction (12). The elevated serum ALP activity in the present study may be due to cytotoxicity, and it was observed in the proximal tubule cells under exposure to gentamicin (13).
One finding of the present study was degenerative alteration in the epithelium of the renal tubules under light microscope, which is in accordance with the study by Deichmann and Keplinger (14). It is supported by the deformation and shrinkage of the nuclei, and also by the loss of microvilli in the epithelial cells of the PCTs observed under electron microscopic assay. Berman et al. (15) reported early pathological changes in the rat kidney, including tubular degeneration, necrosis, and vacuolar degeneration, after 14 days of daily exposure to 40 mg/kg of phenol using gavage dosing. The authors attributed these lesions to vascular stasis in the kidney. Other authors also demonstrated similar renal histological changes in rats after exposure to chemical compounds containing phenol (16,17). This result is in accordance with our data. Although the mechanisms of phenol nephrotoxicity are not clear, hydrophobicity and formation of phenoxyl radicals are noted as possible mechanisms of phenolic cytotoxicity (18). The degenerative alterations mentioned in the present study may be due to phenoxyl-type radicals derived from phenol and their ability to impair epithelial cell membrane integrity (18).

The interstitial lymphoplasmacytic nephritis observed in this study expresses signs of irritability, inflammation, and hypersensitivity to the used toxicant. In relation to this, Robb and Marchevsky reported that inflammation exposes body organs to infection, leading to the release of high amounts of white blood cells (19).

In the present study, phenol exposure could induce deformity in the shape of mitochondria. Mitochondria are the main source of energy supply, with great importance to renal tubules for optimum performance. Inefficiency of renal function (Table 1) and structure (Figures 1-4) may be attributed to mitochondrial destruction.

Regarding the ultrastructural results of the present study, the phenol administration could induce the formation of EEDs in the basement membrane of the glomerulus. It seems that the normality of the basement membrane of the glomerulus is essential for a normal glomerular filtration rate (GFR), and any change in this structure leads to proteinuria (20).

Dilation of the urinary space of the renal corpuscles in phenol-treated mice may be due to basement membrane alterations and PCT epithelial changes, decreasing the functional properties of PCTs and resulting in a GFR decrease and the accumulation of urine in the urinary space.

In conclusion, the significant increases observed in serum CRT and BUN levels, ALP activity, and histopathological changes of the renal tissue in mice exposed to phenol indicate tissue injuries. These findings suggest that treatment with different concentrations of phenol through gavage for 10 consecutive days may cause nephrotoxicity in mice.

References