DETRIMENTAL EFFECTS OF SODIUM NITRITE ON THE KIDNEY OF SWISS ALBINO MICE.

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ABSTRACT: The present study aimed to investigate the possible effects of sodium nitrite toxicity on histological parameters of kidney in the Swiss albino mice at the rate of 50mg/kg bw and 75 mg/kg bw daily in single dose for 120 days. Histopathological examination revealed morphological alterations like lobulation of glomerular tuft. Cellular infiltrates are prominent around bowman's capsule and tubulo-interstitium. Degeneration of tubular epithelial cells and loss of brush border of proximal convoluted tubule are quite evident. On the basis of these findings the study suggest that sodium nitrite has direct toxic effects on the kidney.

Key Words: Sodium nitrite, Swiss albino mice, HistopathologyKidney, Degeneration

INTRODUCTION
Sodium nitrite having the chemical formula NaNO₂ is white to slightly yellow crystalline powder. It has been used as preservative and color stabilizer in meat, poultry and fish products. The sodium nitrite when added to meat gets converted to nitric oxide which combines with myoglobin to form nitric oxide myoglobin (nitrosyl myoglobin) which is a heat stable pigment. The curing contributes flavor to meat. In addition, nitrite curing inhibits the growth of Clostridium and Streptococcus and also lowers the temperature required to kill C. botulinum.

Sodium nitrite commonly used in processed meat cured and smoked meat and fish, root vegetables, some cheese, hamburger and pizza etc. (Handerson and Raskin 1972). Sometime nitrite salts can react with certain amines (derivatives of ammonia) in food to produce nitrosamine many of which are known to cause cancer (Taylor, 1983).

The acute toxicity of sodium nitrite is approximately of ten-fold higher than that of nitrite with LD50 value 214,180,186 mg/kg body weight in mice, rats and rabbits respectively (NIOSH, 1987, Spijier et. al., 1987, Walker, 1990).

The major acute toxic effect of nitrite on the haemopoietic system is the oxidation of hemoglobin into methemoglobin which results into serious impairment of oxygen carrying capacity of blood. (Imaizumi et.al., 1980, Tarburton and Metcalf, 1986). Atef et.al. (1991) reported that nitrate and nitrites are environmental pollutants present in water that may be contributed to the etiology of kidney disease and problem related to immunity in domestic fowl.

MATERIALS AND METHODOLOGY
Male swiss albino mice with average body weight of 30±2 grams were selected for the experimental study. All experimental procedures were conducted as per the guide lines of committee for the purpose of control and supervision of experiments on animals (CPCSEA). The animals were housed at controlled environmental condition 22±2°C, relative humidity 50±10% and 12-hour dark light cycle. Animals were housed in polypropylene cages and allowed free access of food and water. The mice were divided in two groups. There was the control group and treated group. The control group had normal basic structure of kidney. The second group was treated with sodium nitrite 50mg/kg bw and 75mg/kg bw for 120 days. After the experimental period was over the animals were sacrificed for histological studies. The selected organ was dissected and fixed into 10% neutral formalin fixative and tissues were processed and stained with hematoxylin and eosin (H&E) and finally examined under the microscope. The procedures followed are those of Drury and Wallington (1967).

All the experimental data were expressed as mean±SD. The changes in the diameter of different parts of kidney in the treated group were compared to the control and significance analyzed statistically by T-test. Differences were considered significant if P<0.05.
RESULTS

TABLE -1
Diameter of glomerulus (in mm) of Kidneys of mice after oral administration of sodium nitrite.

<table>
<thead>
<tr>
<th>Dose Duration</th>
<th>Control</th>
<th>50mg/kg bw/day Sodium Nitrite</th>
<th>75mg/kg bw/day Sodium Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 DAYS</td>
<td>0.053531 +</td>
<td>0.049399* +</td>
<td>0.049835* +</td>
</tr>
<tr>
<td></td>
<td>+ 0.0036878</td>
<td>+ 0.004</td>
<td>+ 0.0033015</td>
</tr>
</tbody>
</table>

*P<0.05

TABLE -2
Diameter of Bowman’s capsule (in mm) of Kidneys of mice after oral administration of sodium nitrite.

<table>
<thead>
<tr>
<th>Dose Duration</th>
<th>Control</th>
<th>50mg/kg bw/day Sodium Nitrite</th>
<th>75mg/kg bw/day Sodium Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 DAYS</td>
<td>0.082233 +</td>
<td>0.080820 +</td>
<td>0.080597 +</td>
</tr>
<tr>
<td></td>
<td>+ 0.00374165</td>
<td>+ 0.0796241</td>
<td>+ 0.00353553</td>
</tr>
</tbody>
</table>

*P<0.05

TABLE -3
Diameter of proximal convoluted tubules (in mm) of Kidneys of mice after oral administration of sodium nitrite.

<table>
<thead>
<tr>
<th>Dose Duration</th>
<th>Control</th>
<th>50mg/kg bw/day Sodium Nitrite</th>
<th>75mg/kg bw/day Sodium Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 DAYS</td>
<td>0.033736 +</td>
<td>0.035811 +</td>
<td>0.036215* +</td>
</tr>
<tr>
<td></td>
<td>+ 0.00145602</td>
<td>+ 0.028705</td>
<td>+ 0.0018055</td>
</tr>
</tbody>
</table>

*P<0.05

TABLE -4
Diameter of distal convoluted tubules (in mm) of Kidneys of mice after oral administration of sodium nitrite.

<table>
<thead>
<tr>
<th>Dose Duration</th>
<th>Control</th>
<th>50mg/kg bw/day Sodium Nitrite</th>
<th>75mg/kg bw/day Sodium Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 DAYS</td>
<td>0.030646 +</td>
<td>0.03271* +</td>
<td>0.033718* +</td>
</tr>
<tr>
<td></td>
<td>+ 0.0016552</td>
<td>+ 0.0013228</td>
<td>+ 0.0030983</td>
</tr>
</tbody>
</table>

*P<0.05

TABLE -5
Diameter of collecting ducts (in mm) of Kidneys of mice after oral administration of sodium nitrite.

<table>
<thead>
<tr>
<th>Dose Duration</th>
<th>Control</th>
<th>50mg/kg bw/day Sodium Nitrite</th>
<th>75mg/kg bw/day Sodium Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 DAYS</td>
<td>0.026802 +</td>
<td>0.028666 +</td>
<td>0.029146 +</td>
</tr>
<tr>
<td></td>
<td>+ 0.023194</td>
<td>+ 0.0014247</td>
<td>+ 0.012688</td>
</tr>
</tbody>
</table>

*P<0.05
Plate 1a- (H&E) kidney of control mice showing normal glomerulus (G), Bowman's capsule (BC), proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) X 400

Plate 1b kidney of control mice showing normal glomerulus (G), Bowman's capsule (BC) X1000.

Plate 1c kidney of control mice showing normal appearance of proximal convoluted tubules and distal convoluted tubules. The nuclei of renal tubules seen normal in number and shape X 1000.

Plate 2. Treated t sodium nitrite oral dose of 50mg/kg bw for 120 days. It shows necrosis of epithelial cells of bowman’s capsule (BC), pycnotic nuclei (NP) and lobulation of glomerulus (G) more distinctly due to formation of sharp furrow. Degeneration of glomerular mass also observed X1000.
Plate 3. Treated with sodium nitrite oral dose of 50mg/kg bw for 120 days. It shows loss of brush border of proximal convoluted (PCT). Lumen of tubules appeared to be filled with degenerated cytoplasmic debris. Cytoplasmic vacuolization (V) also present. Crowd of nuclei (CN) are visible around renal tubules probably because of splitting of nuclei of renal tubular cell due to toxicity x 1000.

Plate 4. Treated with sodium nitrite oral dose of 75 mg/kg bw for 120 days. It shows lobulation of glomerular tuft. Glomerular capillary tuft appeared clumped. Cellular infiltrate present between tubular epithelial cells are observed. Degeneration of cytoplasm of tubular epithelial cells also seen X1000.

Plate 5. Treated with sodium nitrite oral dose of 75 mg/kg bw for 120 days cellular infiltrate present between tubular epithelial cells are observed. Degeneration of cytoplasm of tubular epithelial cells also seen x1000.

DISCUSSION
In the treated group changes in the different parameters were noted. The deviation from the normal basic structure were observed in different parameters of the kidney. The different sub divisions of kidney showed marked change due to both doses for the particular duration of treatment with sodium nitrite. There was significant decrease in the diameter of glomerulus after treatment with both doses 50 mg/kg bw and 75mg/kg bw sodium nitrite orally per day after 120 days exposure. In histological study necrosis of epithelium of Bowman’s capsule was clearly seen. The parietal epithelium of Bowman’s capsule was degenerated. Vacuoles were prominently observed in the glomerulus. The nuclei of glomerulus appeared to be pycnotic or clumped. The glomerulus is target of toxic effects of number of drugs and chemical agents.
that may cause changes in glomerular morphology. The epithelium of glomerulus undergoes irreversible damage. The capacity for these cell types to repair damaged tissue is restricted as compared to the other cell types such as proximal convoluted tubules. Although glomerulus and bowman's capsule are together referred to as renal corpuscles but they differ greatly in extent of damage as well as repair. Imane et al. (2011) in the study of tartrazine at the doses of 1,2, and 3% for three weeks reported intercapillary sclerosis and atrophy of glomerulus in guinea pigs.

In rats after 200 days treatment with 170mg/kg bw and 340 mg/kg bw sodium nitrite per day showed some changes in liver and kidney weights as well as hemoglobinemia raised heart weight and raised spleen weight (Musil, 1966).

Haider (1986) has reported pathological changes like inflammation condition in glomeruli, disorganization in the epithelioid granular cells to mild or acute level, appearance of unusual gaps inside the Malpighian capsule in his studies. He also reported shrinkage of the glomeruli to various degree.

The kidney of aluminum chloride treated mice showed shrunken glomeruli. Intraglomerular congestion, mesangial hyperplasia and obliteration of the filtration slits. Loss of apical microvilli degeneration of mitochondria and widened rough endoplasmic reticulum were also observed in proximal convoluted tubules of treated animal. (Kahtani, 2010).

There was no significant change observed in the diameter of proximal convoluted tubules after treatment with 50 mg/kg bw sodium nitrite for 120 days. In histological analysis study loss of brush border was observed. The lumen of tubules filled with degenerated cytoplasmic debris. Initiation of cytoplasmic vacuolization was also seen. The diameter of proximal convoluted tubule significantly increased after 120 days treatment with 75 mg/kg bw sodium nitrite per day at P<0.05%. In histological study accumulation of edematous fluid was observed around the PCT seen. Tubular cytolysis was also seen.

The increase in diameter of proximal convoluted tubules, may be attributed to tubular dilatation with flattening of cells. Dilatation of tubular lumina can be explained as resulting from increased intracellular pressure according to Olsen and Solez (1982).

Sodium nitrite caused pathological change in rat kidney (Gojer and Sawant, 1992) and pheasant chick liver and kidney (Storand and Persin, 1983).

There was significant increase in the diameter of distal convoluted after both 50mg/kg bw and 75 mg/kg bw doses of sodium nitrite for 120 days oral treatment at P< 0.05%. In histopathological study necrosis of distal convoluted tubule was seen. Crowd of nuclei were observed around the bowman's capsule and within the tubulointerstitium. Grudzinsky (1991) suggested that 100 ml sodium nitrite acts on the plasma membrane of the enterocyte providing possibilities for producing lability of these membrane which is associated with change transport function.

Abd El Tawab et al. (2003) showed that sodium nitrite caused dilation and congestion of glomerular and peritubular capillaries. The lumen of renal tubules become dilated with degeneration of its cells.

Bulger et al. (1983) reported that administration of mercuric chloride to rats results in few changes in the distal convoluted tubules which may however show some degree of degeneration.

There were no significant changes observed in the diameter of collecting duct after both doses of 50mg/kg bw and 75 mg/kg bw per day treatment with sodium nitrite for 120 days. In histopathological study tubular cytolysis was observed. The collecting ducts appear to be relatively insensitive of most nephrotoxicants. Damage due to tetracyclines may occur in these segments (Hook and Hewitt, 1986).

On the basis of these findings the present study concluded that continuous use of sodium nitrite and also its use in excess quantity is harmful to health.

REFERENCE


14. NIOSH (The national institute for occupational Safety and Health U.S.A) 1987 NIOSH Registry of toxic effects of chemical substances.


