The effect of sodium fluoride administration on hydroxyproline concentration in rat kidneys

Eman Ali Al-Omireeni
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  - d) NaF
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Kidneys are paired, bean-shaped organs situated in a retroperitoneal position on the posterior aspect of the abdominal cavity, on either side of the vertebral column.

The kidney is covered by a fibrous capsule. The outer zone of the kidney is the renal cortex which contains the glomeruli and the inner zone is the medulla.
The nephron is the structural and functional unit of the kidney. Each kidney contains 30,000 to 35,000 nephrons in the rats and about 1 million in humans. Each nephron is made up of a renal corpuscle (glomerulus) and a complex tubular portion, which drains into a unifying tubular system called the collecting duct system.
Hydroxyproline (Hyp)

- Hydroxyproline is a modified amino acid that is derived from proline by post-translation hydroxylation occurring within the peptide chain in biosynthesis of collagen.
- The occurrence of this amino acid is thought to be confined exclusively to collagen, where it is present in the Y position of the Gly-X-Y repeating tripeptide.
Collagen fibers
Forms of Hydroxyproline in the Tissues

1. Free Hydroxyproline.
2. Peptide-bound Hydroxyproline.
3. Protein-bound Hydroxyproline.
4. Soluble collagen hydroxyproline and insoluble collagen hydroxyproline
Collagen content in tissue is determined by measuring its hydroxyproline content.

Breakdown or remodeling of collagen occurs normally in tissues in response to growth or injury. However, during rapid growth and in disease states, the extent of collagen degradation is extensive.
Hydroxyproline from collagen degradation

- 10% excreted in urine
- 90% oxidized in liver to CO$_2$ and urea
Fluoride

- Fluoride is taken mainly in drinking water beside various nutrition products and beverages like tea, drugs, fluoride containing salt.
- Fluoride has both beneficial and detrimental effects on human health, with a narrow range between the intakes associated with its beneficial and adverse health effects.
Aim

- To study the effect of sodium fluoride administration on hydroxyproline/collagen concentration in rat kidneys.
- To study the reported protective effect of magnesium chloride on sodium fluoride induced changes in rat kidney hydroxyproline/collagen.
Materials and Methods
Protocol

1. Injection of sodium fluoride (intraperitoneally)
2. Kidneys removed and homogenized
3. Extraction of different hydroxyproline fractions
4. Determination of hydroxyproline/collagen concentration
All the groups consisted of 6–8 rats of the same age and weight.
The rats are injected with NaF/MgCl\textsubscript{2} through intraperitoneal route.
All the rats were sacrificed 24 hours after the treatment.

Kidneys were taken from rats, washed in buffered saline, frozen immediately in liquid nitrogen and stored at -80°C until processed.

Tissues were homogenized in normal saline (0.85% NaCl). The homogenate was used to determine the free, peptide and protein-bound Hyp.

Tissues were homogenized in 0.45% NaCl to determine the soluble and insoluble collagen Hyp.
Extraction of Free, Peptide-bound and Protein-bound Hyp

- **0.5ml Homogenate + 2ml Cold Ethanol**
  - Centrifuge 3000 rpm, 10 min, 4°C
  - Supernatant
  - Pellet

- **Cold Ethanol Supernatant**
  - +2ML Cold Ethanol
  - Centrifuge 3000 rpm, 10 min, 4°C
  - Supernatant
  - Pellet

- **Pooled Supernatant**
  - Free Hyp

- **Pellet**
  - Peptide bound Hyp
  - Protein bound Hyp
Determination of Free, Peptide-bound and Protein-bound Hyp

Supernatant:
- 1 ml evaporated at 40°C
- 25 µl of 8N NaOH
- Chloramine T 56 mM
- 900 µl, 25 min
- Ehrlich’s Aldehyde
- 1 M, 1 ml, 65°C, 20 min
- Absorbance at 550 nm

Free Hyp:
- 50 µl + 25 µl of 8N NaOH, hydrolyzed
- Chloramine T 56 mM
- 900 µl, 25 min
- Ehrlich’s Aldehyde
- 1 M, 1 ml, 65°C, 20 min
- Absorbance at 550 nm

Peptide bound Hyp:
- 50 µl
- + 25 µl of 8N NaOH
- Hydrolyzed
- Chloramine T 56 mM
- 900 µl, 25 min
- Ehrlich’s Aldehyde
- 1 M, 1 ml, 65°C, 20 min
- Absorbance at 550 nm

Protein bound Hyp:
- Pellet
- 25 µl of 8N NaOH
- Hydrolyzed
- Chloramine T 56 mM
- 900 µl, 25 min
- Ehrlich’s Aldehyde
- 1 M, 1 ml, 65°C, 20 min
- Absorbance at 550 nm
Extraction of soluble in soluble collagen Hyp

1. 3ml Homogenate
   - centrifuge 13000rpm 60 min 4°C
   - 3ml Supernatant
   - +4ml 100% ethanol centrifuge 3000rpm 10 min, 4°C
   - +2ml 80% Ethanol
   - +2ml 100% Ethanol
   - +2ml Diethyl ether
   - +2ml 100% Ethanol
   - 2ml Ethanol + Diethyl ether 1:2

2. pellet
   - 2ml DDH2O
   - 2ml NaCl centrifuge 3000rpm 10 min, 4°C
   - +2ml 100% Ethanol
   - +2ml Diethyl ether
   - +2ml 100% Ethanol
   - 2ml Ethanol + Diethyl ether 1:2

Soluble collagen Hyp

Insoluble collagen Hyp
Determination of soluble in soluble collagen Hyp

1. **Pellet from the homogenate**
   - Gelatinize + DD H2O
   - 3 hours, 124°C

2. **Pellet from the supernatant**
   - Gelatinize + DD H2O
   - 3 hours, 124°C

3. **Filtration**

4. **Chloramine T 56mM**
   - 900 µl, 25 min

5. **Ehrlich’s Aldehyde**
   - 1M, 1 ml, 65°C, 20 min

6. **Hydrolyzed in 8N NaOH**

7. **Filtration**

8. **Absorbance at 550nm**

9. **Insoluble collagen Hyp**

10. **Soluble collagen Hyp**
Results

- Standard graph
- Effect of different doses of NaF on renal hydroxyproline/collagen in rats
- Effect of MgCl₂ on NaF induced changes in renal hydroxyproline/collagen in rats
Standard graph
Standard graph of hydroxyproline

Absorbance at 550 nm

Concentration of hydroxyproline (μg)
Effect of different doses of NaF on kidney hydroxyproline/collagen in rats*.

Effect of different doses of NaF on survival in rats

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Dose of NaF mg/kg body weight</th>
<th>Number of rats injected with NaF</th>
<th>Number of rats Survived</th>
<th>Percentage survival</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>12</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>12</td>
<td>12</td>
<td>100</td>
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<td>3</td>
<td>5</td>
<td>12</td>
<td>12</td>
<td>100</td>
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<tr>
<td>4</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>15</td>
<td>10</td>
<td>67%</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>15</td>
<td>4</td>
<td>27%</td>
</tr>
</tbody>
</table>
Effect of NaF treatment on body weight, kidney weight and kidney protein in rats

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Experimental Groups (NaF treated)</th>
<th>Body Weight (grams)</th>
<th>Kidney Weight (grams)</th>
<th>Kidney protein (mg/gm fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>228.3 ± 3.20</td>
<td>1.20 ± 0.16</td>
<td>167.3 ± 37.96</td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>235.2 ± 7.55</td>
<td>1.15 ± 0.12 ns</td>
<td>154.4 ± 43.56 ns</td>
</tr>
<tr>
<td>3</td>
<td>5 mg/kg</td>
<td>231.8 ± 15.35 ns</td>
<td>1.14 ± 0.14 ns</td>
<td>74.33 ± 8.98 ***</td>
</tr>
<tr>
<td>4</td>
<td>10 mg/kg</td>
<td>183.8 ± 16.92 ***</td>
<td>1.09 ± 0.17 ns</td>
<td>113.7 ± 22.64 *</td>
</tr>
<tr>
<td>5</td>
<td>20 mg/kg</td>
<td>250.0 ± 0.09 **</td>
<td>1.04 ± 0.04 ns</td>
<td>159.9 ± 15.61 *</td>
</tr>
<tr>
<td>6</td>
<td>30 mg/kg</td>
<td>270.0 ± 0.00 ***</td>
<td>1.06 ± 0.1 ns</td>
<td>59.33 ± 6.64 ***</td>
</tr>
</tbody>
</table>
**Effect of NaF treatment on organosomatic index in rat kidney**

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Dose of NaF (mg/kg body weight)</th>
<th>Organ somatic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>0.61 ± 0.05&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.50 ± 0.07&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0.60 ± 0.07&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.41 ± 0.02&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>0.40 ± 0.04&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Effect of NaF treatment on serum biochemical parameters in rats

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control</th>
<th>Placebo</th>
<th>5 mg/kg body weight</th>
<th>10 mg/kg body weight</th>
<th>20 mg/kg body weight</th>
<th>30 mg/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>33.5 ± 3.12</td>
<td>34.50 ± 3.32ns</td>
<td>40.75 ± 4.92ns</td>
<td>62.0 ± 9.31***</td>
<td>57.0 ± 8.83***</td>
<td>30.50 ± 4.44*</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>17.75 ± 3.00</td>
<td>17.25 ± 2.22ns</td>
<td>19.75 ± 2.36ns</td>
<td>17.25 ± 1.5*</td>
<td>30.00 ± 6.05**</td>
<td>23.75 ± 6.19ns</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.37 ± 0.07</td>
<td>0.40 ± 0.06ns</td>
<td>0.55 ± 0.05*</td>
<td>0.36 ± 0.06ns</td>
<td>0.51 ± 0.03ns</td>
<td>0.47 ± 0.09ns</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.33 ± 0.21</td>
<td>1.28 ± 0.52ns</td>
<td>1.40 ± 0.27ns</td>
<td>1.63 ± 0.15ns</td>
<td>0.70 ± 0.14ns</td>
<td>1.50 ± 0.52ns</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.56 ± 0.45</td>
<td>3.71 ± 0.33ns</td>
<td>3.54 ± 4.50ns</td>
<td>3.95 ± 0.17ns</td>
<td>3.10 ± 0.34ns</td>
<td>2.66 ± 0.21ns</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>6.20 ± 0.45</td>
<td>6.44 ± 0.43ns</td>
<td>7.35 ± 0.38ns</td>
<td>7.63 ± 0.60*</td>
<td>5.55 ± 0.77ns</td>
<td>5.02 ± 0.59*</td>
</tr>
</tbody>
</table>
Effect of NaF treatment on serum electrolytes concentration in rats

<table>
<thead>
<tr>
<th>Serum Electrolytes</th>
<th>Control</th>
<th>Placebo</th>
<th>5 mg/kg body weight</th>
<th>10 mg/kg body weight</th>
<th>20 mg/kg body weight</th>
<th>30 mg/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/l)</td>
<td>143.8 ± 4.35</td>
<td>144.5 ± 6.46&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>148.8 ± 6.5&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>142.8 ± 3.1&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>139.5 ± 2.52&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>136.0 ± 4.24&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>5.58 ± 0.96</td>
<td>6.50 ± 3.48&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>6.20 ± 0.51&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>11.75 ± 0.87&lt;sup&gt;***&lt;/sup&gt;</td>
<td>4.63 ± 0.17&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.68 ± 0.50&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>12.34 ± 0.66</td>
<td>12.82 ± 0.23&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>12.75 ± 1.20&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>11.99 ± 0.25&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>9.49 ± 0.13&lt;sup&gt;***&lt;/sup&gt;</td>
<td>8.55 ± 0.19&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Magnesim (mg/dl)</td>
<td>2.45 ± 0.04</td>
<td>2.54 ± 0.32&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>3.21 ± 0.47&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.44 ± 0.22&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2.44 ± 0.12&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2.34 ± 0.45&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphorous (mg/dl)</td>
<td>10.24 ± 1.64</td>
<td>10.24 ± 2.23&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>12.42 ± 2.43&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>10.80 ± 0.86&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>6.42 ± 0.61&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.79 ± 0.94&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Effect of NaF treatment on serum alkaline phosphatase in rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Alkaline Phosphatase (U/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Placebo</td>
<td>ns</td>
</tr>
<tr>
<td>5 mg</td>
<td>ns</td>
</tr>
<tr>
<td>10 mg</td>
<td>ns</td>
</tr>
<tr>
<td>20 mg</td>
<td>ns</td>
</tr>
<tr>
<td>30 mg</td>
<td></td>
</tr>
</tbody>
</table>

* **
ns
ns
ns
Effect of NaF treatment on different hydroxyproline fractions in rat kidneys

<table>
<thead>
<tr>
<th>Experimental Groups (NaF treated)</th>
<th>Free Hyp (µg/gm fresh tissue)</th>
<th>Peptide-bound Hyp (mg/gm fresh tissue)</th>
<th>Protein-bound Hyp (mg/gm fresh tissue)</th>
<th>Total Hyp (mg/gm fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Placebo 5mg/kg body weight</td>
<td>329.3 ± 54.65 276.8 ± 24.66&lt;sup&gt;ns&lt;/sup&gt; 283.5 ± 50.7&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>11.12 ± 0.92 5.92 ± 3.2&lt;sup&gt;*&lt;/sup&gt; 8.91 ± 2.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.048 ± 0.15 1.22 ± 0.37&lt;sup&gt;ns&lt;/sup&gt; 2.76 ±0.40&lt;sup&gt;***&lt;/sup&gt;</td>
<td>12.32 ± 2.76 6.86 ± 0.59&lt;sup&gt;**&lt;/sup&gt; 11.76± 3.07&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 mg/kg body weight</td>
<td>125.3±15.94&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1.61± 0.9&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1.26 ± 0.23&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2.93 ±0.75&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 mg/kg body weight</td>
<td>245.5 ± 40.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.89 ± 0.41&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.49 ± 0.21&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2.98 ±0.67&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>30 mg/kg body weight</td>
<td>333.2 ± 49.5&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.30 ± 0.25&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.308 ± 0.29&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2.62 ±0.49&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Effect of NaF on soluble collagen hydroxyproline in rat kidneys

Dose of Sodium Fluoride (mg/kg body weight)

Soluble collagen Hyp (μg/gram tissue)

- Control
- Placebo
- 5 mg
- 10 mg
- 20 mg
- 30 mg

** ns
* ns
ns ns
Effect of NaF on insoluble collagen hydroxyproline in rat kidneys

![Graph showing the effect of NaF on insoluble collagen hydroxyproline in rat kidneys. The x-axis represents the dose of sodium fluoride (mg/kg body weight), ranging from 0 to 30 mg. The y-axis represents the insoluble collagen Hyp (µg/gram tissue), ranging from 0 to 300 µg/gram tissue. The graph includes data for control, placebo, and doses of 5 mg, 10 mg, 20 mg, and 30 mg. Significant differences are indicated with an asterisk (*).]
Effect of NaF on total collagen hydroxyproline in rat kidneys

Experimental Groups

- Control
- Placebo
- 5 mg
- 10 mg
- 20 mg
- 30 mg

Total collagen (mg/g tissue)

*** ns *** *** ***
Electron micrograph of kidney from control animal
Control
Control
Electron micrograph of kidney collagen in rats injected with 5, 10, 20 and 30 mg/kg body weight of NaF*

5mg/kg body weight NaF
10mg/kg body weight NaF
20mg/kg body weight NaF
30mg/kg body weight NaF
Effect of MgCl$_2$ on NaF induced changes in renal hydroxyproline/collagen in rats*

Effect of MgCl$_2$ and NaF treatment on body weight, kidney weight and protein content in rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Body weight (grams)</th>
<th>Kidney weight (grams)</th>
<th>Kidney protein (mg/gram tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>228.33 ± 3.20</td>
<td>1.07 ± 0.97</td>
<td>150.1 ± 37.5</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>200.83 ± 13.67**</td>
<td>0.97 ± 0.10$^{\text{ns}}$</td>
<td>45.61 ± 2.04***</td>
</tr>
<tr>
<td>NaF</td>
<td>183.83 ± 16.91***</td>
<td>1.09 ± 0.17$^{\text{ns}}$</td>
<td>113.7 ± 22.64*</td>
</tr>
<tr>
<td>MgCl$_2$ + NaF</td>
<td>178.83 ± 7.78***</td>
<td>0.76 ± 0.06$^{\text{ns}}$</td>
<td>54.93 ± 2.88***</td>
</tr>
</tbody>
</table>
Effect of MgCl\textsubscript{2} and NaF treatment on organosomatic index in rat kidney

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Groups</th>
<th>Organ somatic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.58 ± 0.72</td>
</tr>
<tr>
<td>2</td>
<td>NaF</td>
<td>0.62 ± 0.11\textsuperscript{ns}</td>
</tr>
<tr>
<td>3</td>
<td>MgCl\textsubscript{2}</td>
<td>0.49 ± 0.04\textsuperscript{ns}</td>
</tr>
<tr>
<td>4</td>
<td>NaF+MgCl\textsubscript{2}</td>
<td>0.41 ± 0.05\textsuperscript{ns}</td>
</tr>
</tbody>
</table>
Effect of MgCl$_2$ and NaF treatment on serum biochemical parameters in rats

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control</th>
<th>MgCl$_2$</th>
<th>NaF</th>
<th>MgCl$_2$ + NaF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>33.5 ± 3.12</td>
<td>42.50 ± 4.80$^{ns}$</td>
<td>62.0 ± 9.31$^{***}$</td>
<td>48.75 ± 4.65$^{*}$</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>17.75 ± 3.30</td>
<td>22.75 ± 2.75$^{ns}$</td>
<td>17.25 ± 1.5$^{ns}$</td>
<td>22.75 ± 2.22$^{ns}$</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.37 ± 0.07</td>
<td>0.47 ± 0.02$^{ns}$</td>
<td>0.36 ± 0.06$^{ns}$</td>
<td>0.46 ± 0.03$^{ns}$</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.33 ± 0.21</td>
<td>0.85 ± 0.13$^{*}$</td>
<td>1.63 ± 0.15$^{ns}$</td>
<td>1.5 ± 0.29$^{ns}$</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.56 ± 0.45</td>
<td>3.94 ± 0.28$^{ns}$</td>
<td>3.97 ± 0.15$^{ns}$</td>
<td>4.32 ± 0.15$^{*}$</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>6.20 ± 0.45</td>
<td>7.62 ± 0.22$^{***}$</td>
<td>7.63 ± 0.60$^{***}$</td>
<td>7.28 ± 0.23$^{**}$</td>
</tr>
</tbody>
</table>
Effect of MgCl$_2$ and NaF treatment on serum electrolytes concentration in rats

<table>
<thead>
<tr>
<th>Serum Electrolytes</th>
<th>Control</th>
<th>MgCl$_2$</th>
<th>NaF</th>
<th>MgCl$_2$ + NaF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/I)</td>
<td>142.6 ± 4.56</td>
<td>138.8 ± 3.77ns</td>
<td>142.8 ± 3.0ns</td>
<td>132.2 ± 2.95**</td>
</tr>
<tr>
<td>Potassium (mEq/I)</td>
<td>5.58 ± 0.96</td>
<td>5.6 ± 0.36ns</td>
<td>11.75 ± 0.87***</td>
<td>5.85 ± 0.35ns</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>12.34 ± 0.66</td>
<td>10.81 ± 0.12**</td>
<td>11.99 ± 0.25ns</td>
<td>10.78 ± 0.45**</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>2.45 ± 0.05</td>
<td>2.37 ± 0.14ns</td>
<td>2.44 ± 0.22ns</td>
<td>3.05 ± 0.20**</td>
</tr>
<tr>
<td>Phosphorous (mg/dl)</td>
<td>10.24 ± 1.64</td>
<td>8.78 ± 0.31ns</td>
<td>10.80 ± 0.86ns</td>
<td>11.36 ± 0.60ns</td>
</tr>
</tbody>
</table>
Effect of MgCl$_2$ and NaF on alkaline phosphatase in rat serum

![Graph showing the effect of MgCl$_2$ and NaF on alkaline phosphatase in rat serum. The x-axis represents the experimental groups, and the y-axis represents the Alkaline Phosphatase (U/I). The graph indicates that MgCl$_2$ and NaF alone and in combination do not significantly affect alkaline phosphatase levels (ns).]
Effect of MgCl$_2$ and NaF treatment on various hydroxyproline fractions in rat kidneys

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Free Hyp (µg/gm fresh tissue)</th>
<th>Protein-bound Hydroxyproline (mg/gm fresh tissue)</th>
<th>Peptide-bound Hydroxyproline (mg/gm fresh tissue)</th>
<th>Total Hydroxyproline (mg/gm fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>329.3 ± 54.65</td>
<td>1.04 ± 0.15</td>
<td>11.29 ± 0.88</td>
<td>12.67 ± 0.96</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>149.6 ± 22.77***</td>
<td>1.73 ± 0.17***</td>
<td>1.99 ± 0.47***</td>
<td>3.87 ± 0.48***</td>
</tr>
<tr>
<td>NaF</td>
<td>125.3 ± 15.94***</td>
<td>1.26 ± 0.23 ns</td>
<td>1.61 ± 0.91***</td>
<td>3.44 ± 0.79***</td>
</tr>
<tr>
<td>MgCl$_2$ + NaF</td>
<td>218.1 ± 11.16***</td>
<td>2.41 ± 0.33***</td>
<td>6.67 ± 1.23***</td>
<td>9.19 ± 1.21***</td>
</tr>
</tbody>
</table>
Effect of MgCl₂ and NaF on soluble collagen hydroxyproline in rat kidneys

Experimental Groups

Soluble collagen Hyp (µg/gram tissue)

- Control
- MgCl₂
- NaF
- MgCl₂ + NaF
Effect of MgCl$_2$ and NaF on insoluble collagen hydroxyproline in rat kidneys

Experimental Groups

Insoluble collagen Hyp ($\mu$g/gram tissue)

Control
MgCl$_2$
NaF
MgCl$_2$+NaF

ns

ns

ns

Experimental Groups

Insoluble collagen Hyp ($\mu$g/gram tissue)
Effect of MgCl₂ and NaF on total collagen hydroxyproline in rat kidneys

Experimental Groups

Total collagen (mg/gram tissue)
Electron micrograph of kidneys from rats treated with 30 mg/kg body weight of MgCl₂ alone and followed by NaF 10mg/kg body weight /24 hours, 30 minutes after MgCl₂ injection*

30mg/kg body weight MgCl\textsubscript{2}
30mg/kg body weight MgCl₂ Followed by NaF 10mg/kg body weight, 30 minutes after MgCl₂ injection
The hydroxyproline content was expressed as mean ± SD µg/gram fresh tissue. Hydroxyproline levels in various groups were compared using one-way ANOVA analysis followed by Tukey’s multiple comparison test.
These results suggest that NaF disrupts the connective tissue matrix of the kidneys.

Though MgCl₂ has been reported to be protective against the toxic effect of NaF it had no significant effect on NaF induced changes in kidney hydroxyproline content.

Pretreatment of rats with MgCl₂ however restored insoluble collagen hydroxyproline to near normal levels though it had no significant effect on soluble collagen hydroxyproline.
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Thank you for your attention.