Effectiveness of Green Tea Tannin on Rats with Chronic Renal Failure

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The effects of green tea tannin on nephrectomized rats were examined. There were increases in blood urea nitrogen, serum creatinine, and urinary protein, and a decrease in creatinine clearance in the nephrectomized control rats, whereas better results for these parameters were obtained in rats given green tea tannin after nephrectomy, demonstrating a suppressed progression of the renal failure. When the renal parenchyma was partially resected, the remnant kidney showed a decrease in the activity of radical scavenger enzymes. Green tea tannin, however, was found to lighten the kidney under such oxidative stress. Mesangial proliferation and glomerular sclerotic lesions, which were conspicuous in the rats that were not given green tea tannin after nephrectomy, were also relieved.

Key words: green tea; tannin; chronic renal failure; rat

It is known that once chronic renal failure becomes established, the process is irreversible and follows a progressive course to a greater or lesser degree, ultimately leading to cessation of the renal function. Although the mechanism for this progressive course has not yet been fully elucidated, it has been pointed out that glomerular disorders are mainly responsible. We have previously demonstrated that green tea tannin inhibited mesangial cell proliferation, a noted feature of glomerular disorders. These results suggested that this compound had some influence on the renal function. In the present study, to determine its application for treating renal disease, the effect of green tea tannin was investigated on nephrectomized rats, an important experimental model of non-inflammatory renal failure that is useful for investigating the mechanisms responsible for the development and progression of glomerular disorders.

Materials and Methods

Animals. Male Wistar rats were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan).

Green tea tannin. The tea tannin used in this study was Sunphenon (Tarui Kagaku Co., Yokkaichi, Japan), which was prepared from a hot-water extract of green tea, as reported previously. It was composed mainly of (−)-epigallocatechin 3-O-gallate (18.0%), (−)-gallocatechin 3-O-gallate (11.6%), (−)-epicatechin 3-O-gallate (4.6%), (−)-epygallocatechin (15.0%), (+)-gallocatechin (14.8%), (−)-epicatechin (7.0%), and (+)-catechin (3.5%).

Experimental design. Rats weighing about 200 g underwent resection of 1/2 of the left kidney and total excision of the right kidney with an interoperative interval of 12 to 14 days. The blood urea nitrogen level of the rats was determined after their recovery from the operation, and they were divided into three groups to avoid any intergroup difference in the blood urea nitrogen level. The first group was given water, while the other two groups were orally given green tea tannin at 10 or 20 mg kg of body weight/day for 80 consecutive days as drinking water. The dose was adjusted by regulating the concentration in relation to the water consumption. To ensure that the food intake was almost constant among the three groups, the animals were raised on a pair-feeding schedule. Urea nitrogen, creatinine (Cr) in the serum and/or urine, and urinary protein excretion were determined every 20 days during the administration period. At the 80th day, the residual kidney was completely perfused with precooled saline and removed.

Determination of blood and urine samples. Urea nitrogen and Cr were determined by using commercial reagents (BUN Kainos and CRE-EN Kainos obtained from Kainos Laboratories, Tokyo, Japan). Protein was measured according to the biuret method. The creatinine clearance (Ccr), an effective index for expressing the glomerular filtration rate, was calculated on the bases of urinary Cr, serum Cr, urine volume, and body weight by the following equation:

\[
C_{cr} (\text{ml min kg body weight}) = \frac{\text{urinary Cr (mg dl)} \times \text{urine volume (ml)}}{\text{serum Cr (mg dl)}} \times \frac{1000 \times 1}{\text{body weight (g)} \times 1440 \text{ (min)}}
\]

Enzyme assay. A portion of each well-perfused kidney was homogenized with a 4-fold volume of iced physiological saline to determine the activity of enzymes in the homogenate. Superoxide dismutase (SOD) activity was assayed by the nitroblue tetrazolium method, and catalase activity was determined in terms of the decrease in the amount of hydrogen peroxide (H₂O₂) by glutathione peroxidase (GSH-Px) activity was obtained by colorimetry with 2-nitro-5-thiobenzoic acid, a compound produced through the reaction of glutathione and 5,5'-dithiobis (2-nitrobenzoic acid).

Determination of protein in the homogenate. Protein was determined by the method of Lowry et al., using bovine serum albumin as a standard.

Histopathological examination. The other part of the renal tissues was fixed in Bouin's solution, embedded in paraffin, and cut into thin sections. These sections were stained with hematoxylin-eosin, periodic acid Schiff or periodic acid methenamine silver stain, and observed by optical microscopy. Mesangial proliferation was rated as normal, slight, moderate, or severe. The proportion of sclerotic lesions in each glomerulus was rated as grade 0-4, using the method of Raj et al., where grade 1 represents the involvement of up to 25% of the glomerulus, and grade 4 represents the sclerosis of 75-100% of the glomerulus. The glomerular sclerosis index was obtained by averaging the scores for all glomeruli from each rat.

Statistics. Results are presented as mean ± S.E. for 6 rats, all data being analyzed for statistical significance by using Dunnett's test.

Results

Body weight and urine volume

The changes in body weight and urine volume during the experimental period are summarized in Table I. There were no significant differences in body weight change between the control and green tea tannin-treated groups, nor were there any significant differences in urine volume.
Effectiveness of Tannin on Rats with Chronic Renal Failure

Table I. Effect of Green Tea Tannin on Body Weight and Urine Volume

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Dose (mg kg B.W. day)</th>
<th>Body wt. (g)</th>
<th>Urine vol. (ml day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>291.8 ± 3.6</td>
<td>35.2 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>294.0 ± 3.4</td>
<td>36.1 ± 1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>285.2 ± 4.9</td>
<td>35.7 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>338.6 ± 5.8</td>
<td>35.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>348.8 ± 4.6</td>
<td>36.9 ± 2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>345.2 ± 2.6</td>
<td>36.9 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>364.7 ± 5.0</td>
<td>35.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>372.0 ± 6.0</td>
<td>36.3 ± 3.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>374.4 ± 6.7</td>
<td>37.3 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>395.6 ± 4.8</td>
<td>38.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>403.2 ± 5.6</td>
<td>35.6 ± 3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>405.6 ± 7.7</td>
<td>38.8 ± 2.2</td>
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<tr>
<td>80</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>421.2 ± 9.7</td>
<td>42.9 ± 2.5</td>
<td></td>
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<tr>
<td></td>
<td>Green tea tannin</td>
<td>436.2 ± 8.6</td>
<td>37.3 ± 4.3</td>
<td></td>
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<tr>
<td></td>
<td>Green tea tannin</td>
<td>434.8 ± 10.5</td>
<td>40.2 ± 3.6</td>
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</tr>
</tbody>
</table>

Table II. Effect of Green Tea Tannin on Blood Urea Nitrogen

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Dose (mg kg B.W. day)</th>
<th>BUN (mg dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>42.3 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>43.2 ± 2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>41.6 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>39.7 ± 3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>28.7 ± 1.3*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>30.5 ± 1.4*</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>40.2 ± 3.9</td>
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</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>31.7 ± 1.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>32.1 ± 2.2*</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>42.8 ± 4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>34.0 ± 2.8*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>30.2 ± 1.9*</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>51.8 ± 2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>36.9 ± 3.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>40.2 ± 3.6*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal rats</td>
<td>16.4 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.01; *p < 0.001; i.e., significantly different from each nephrectomized control value.

Blood urea nitrogen

Table II shows the blood urea nitrogen data obtained during the period of feeding from the nephrectomized rats with and without green tea tannin administration. In the nephrectomized control rats, the blood urea nitrogen level was significantly increased to reach 51.8 mg dl at 80 days (16.4 mg dl in normal rats). In contrast, in the nephrectomized rats given green tea tannin at a daily dose of 10 mg/kg of body weight, the level was 28.7 mg dl at 20 days, 34.0 mg dl at 60 days, and 36.9 mg dl at 80 days, showing suppression of the incipient increase in blood urea nitrogen. A further increase in the dose to 20 mg did not produce any further suppressive effect on the blood urea nitrogen level.

Serum Cr

The serum Cr level in the nephrectomized control rats increased gradually to reach 0.77 mg dl at 20 days, 0.84 mg dl at 40 days, and 1.05 mg dl at 80 days, reflecting chronic progressive uremia, as shown in Table III. The administration of green tea tannin at a dose of 10 mg kg of body weight/day for 40 days reduced the serum Cr level significantly from 0.84 to 0.73 mg dl. After 80 days, the Cr level was also significantly reduced in the rats given the green tea tannin at a dose of 10 mg. A further increase in the dose to 20 mg produced a further decrease in the Cr level. Oral administration of 20 mg of green tea tannin for 20 days caused a 23% decrease in the level of Cr as compared with that in the control rats. On days 40, 60, and 80, the Cr level was significantly reduced by 17%, 17%, and 18%, respectively, compared with the corresponding control values.
Table IV shows the effect of green tea tannin on Ccr, after administering the oral doses. The Ccr value in the nephrectomized control rats decreased gradually to reach 2.79 ml/min/kg of body weight at 40 days, 2.62 ml at 60 days, and 1.91 ml at 80 days. In contrast, the Ccr value in the rats given 10 and 20 mg of green tea tannin for 20 days increased from 2.73 ml/min/kg of body weight to 3.43 ml/min/kg of body weight at the 10-mg level (a 26% change, \( p < 0.001 \)), and from 2.73 ml/min/kg of body weight to 4.22 ml/min/kg of body weight at the 20-mg level (a 55% change, \( p < 0.001 \)). Similarly, the Ccr value in the rats given the green tea tannin orally for 80 days showed a marked increase at both the 10 and 20 mg dosage levels as compared with that in the control rats, as shown in Table IV.

### Table IV. Effect of Green Tea Tannin on Creatinine Clearance

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Dose (mg kg B.W. day)</th>
<th>Ccr (ml min kg B.W.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.18 ± 0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>4.11 ± 0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>4.21 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.73 ± 0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>3.43 ± 0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>4.22 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.79 ± 0.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>3.66 ± 0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>3.76 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.62 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>3.00 ± 0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>3.04 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.91 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>2.65 ± 0.13</td>
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<tr>
<td></td>
<td>Green tea tannin</td>
<td>2.80 ± 0.15</td>
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</tr>
<tr>
<td></td>
<td>Normal rats</td>
<td>5.43 ± 0.48</td>
<td></td>
</tr>
</tbody>
</table>

\* \( p < 0.05 \); \* \( p < 0.01 \); \* \( p < 0.001 \); i.e., significantly different from each nephrectomized control value.

### Table V. Effect of Green Tea Tannin on Urinary Protein Excretion

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Dose (mg kg B.W. day)</th>
<th>Protein (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>34.0 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>10</td>
<td>32.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>20</td>
<td>36.1 ± 1.2</td>
</tr>
<tr>
<td>20</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>33.6 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>10</td>
<td>25.2 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>20</td>
<td>26.7 ± 3.3</td>
</tr>
<tr>
<td>40</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>51.6 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>10</td>
<td>32.8 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>20</td>
<td>32.6 ± 5.3</td>
</tr>
<tr>
<td>60</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>50.4 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>10</td>
<td>34.3 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>20</td>
<td>32.2 ± 4.0</td>
</tr>
<tr>
<td>80</td>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>51.1 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>10</td>
<td>29.2 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>Green tea tannin</td>
<td>20</td>
<td>33.8 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Normal rats</td>
<td></td>
<td>9.2 ± 0.3</td>
</tr>
</tbody>
</table>

\* \( p < 0.01 \); \* \( p < 0.001 \); i.e., significantly different from each nephrectomized control value.

### Table VI. Effect of Green Tea Tannin on the Activities of Reactive Oxygen Species-scavenging Enzymes in Rats after Excision of 3.4 of Their Kidney Volume

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg kg B.W. day)</th>
<th>SOD (U/mg of protein)</th>
<th>Catalase (U/mg of protein)</th>
<th>GSH-Px (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrectomized rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>8.75 ± 0.40</td>
<td>142.7 ± 11.8</td>
<td>69.63 ± 2.02</td>
</tr>
<tr>
<td>Green tea tannin</td>
<td>10</td>
<td>10.68 ± 0.48°</td>
<td>213.2 ± 13.9°</td>
<td>71.91 ± 3.41°</td>
</tr>
<tr>
<td>Green tea tannin</td>
<td>20</td>
<td>11.66 ± 0.54°</td>
<td>224.4 ± 10.9°</td>
<td>76.97 ± 3.15°</td>
</tr>
<tr>
<td>Normal rats</td>
<td></td>
<td>18.33 ± 1.00</td>
<td>225.9 ± 8.7</td>
<td>85.12 ± 3.95</td>
</tr>
</tbody>
</table>

SOD, superoxide dismutase; GSH-Px, glutathione peroxidase. \* \( p < 0.05 \); \* \( p < 0.01 \); \* \( p < 0.001 \); i.e., significantly different from each nephrectomized control value.
being 52% lower for SOD activity, 37% lower for catalase activity, and 18% lower for GSH-Px activity. However, the activities of both SOD and catalase were higher in the rats given green tea tannin at both the 10- and 20-mg dosage levels for 80 days after nephrectomy. As shown in Table VI, the SOD activity was significantly increased by 22% and 33% in the rats given 10 and 20 mg of green tea tannin, respectively. Similarly, green tea tannin significantly increased the catalase activity from 142.7 to 213.2 U/mg of protein at the 10-mg level (a 49% change, \( p < 0.001 \)) and from 142.7 to 224.4 U/mg protein at the 20 mg level (a 57% change, \( p < 0.001 \)). The GSH-Px activity was significantly increased in the rats given green tea tannin at a dose of 20 mg/kg of body weight.

### Histopathological Evaluation of the Kidney

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Green tea tannin (10 mg)</th>
<th>Green tea tannin (20 mg)</th>
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</thead>
<tbody>
<tr>
<td>Degree of mesangial proliferation</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Slight</td>
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<td>3</td>
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</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>3</td>
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</tr>
<tr>
<td>Severe</td>
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</tbody>
</table>

\*Glomerular sclerosis index \(1.59 \pm 0.18\) \(1.24 \pm 0.12^*\) \(1.15 \pm 0.13^*\)

\*\(p < 0.01\); i.e., significantly different from the control value.

**Histological findings**

As shown in Table VII, no rats in control group showed normal proliferation of the mesangium; the proliferation being rated as slight for 1 rat, moderate for 4, and severe for 1. In the nephrectomized group given green tea tannin

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Fig. Photomicrographs of the Glomeruli Obtained from Rats in the Control (Upper Panel) and Green Tea Tannin-treated (Lower Panel) Groups (\(1 \times 50\)).
at a dose of 10 mg kg of body weight, there were 3 rats with slight or moderate proliferation, while no rats showed normal or severe proliferation. Although the 20-mg group also contained no rats exhibiting normal proliferation, slight proliferation was found in as many as 4 rats, and 2 rats showed moderate proliferation. The glomerular sclerosis index was 1.59 ± 0.18 in the control group, and this was reduced to 1.24 ± 0.12 in the 10-mg group and 1.15 ± 0.13 in the 20-mg group. The typical glomerular morphology seen in the examined kidneys is illustrated in Fig.

**Discussion**

Chronic glomerulonephritis, and hypertension-induced nephrosclerosis, chronic pyelonephritis and diabetic nephropathy have been cited as diseases underlying chronic renal failure. Among them, the most frequently occurring is chronic glomerulonephritis, which is histologically characterized by proliferation of the mesangial cells. Therefore, the mechanism for such proliferation has been extensively studied. The deep involvement of factors derived from the platelet or monocyte series or phagocytes in the progression and aggravation of glomerulonephritis has previously been suggested, and various proliferation-promoting factors produced by these cells have been identified. On the other hand, there are insufficient data on those factors inhibiting the proliferation of mesangial cells; the atrial natriuretic factor, heparin and transforming growth factor beta are limited examples of known inhibitory factors. In a previous study, we found that green tea tannin inhibited the proliferation of mesangial cells and speculated that tannin affects the regulation of the glomerular filtration rate, probably by influencing the physiological function of mesangial cells, i.e., contraction and relaxation of glomeruli. Although we obtained good effects by using cultured mesangial cells, their action must be elucidated further to increase their effectiveness. Therefore, the effect of green tea tannin was examined on nephrectomized rats, a widely used animal model for investigating the progression of glomerular disorders.

Since Brenner's group had demonstrated by the micro-puncture method that the glomerular disorder in nephrectomized model animals was closely related to glomerular hemodynamics, this model has been considered important for investigating the onset and progression of glomerular disorders. Its importance lies in its provision of hypotheses for a key issue in nephrology, the onset and progression of glomerular diseases. In the present study, to analyse the effects of green tea tannin on renal tissue lesions, we focused on the degree of mesangial proliferation and the glomerular sclerosis index, which revealed that renal failure was advanced in rats that had undergone excision of their kidney volume. Nephrectomized rats orally given green tea tannin exhibited milder lesions. This effect was more prominent in those rats given 20 mg of green tea tannin than in those given 10 mg. The decrease in Cₖₒₘ value under conditions of renal failure was also significantly increased after the administration of green tea tannin, suggesting that green tea tannin inhibited the proliferation of mesangial cells, a phenomenon which became increasingly evident as renal failure advanced, while maintaining the filtering function of the glomeruli.

The same action has also been found in Rhei Rhizoma, which is rich in condensed-type tannin like green tea tannin, and in its active component, epigallocatechin 3-O-gallate. Such an inhibitory action on the proliferation of mesangial cells has also been found with magnesium lithospermate B, a tetramer of caffeic acid linked with a magnesium salt, suggesting that this newly found activity is common to phenol compounds above a certain molecular weight.

It is known that a subtotal nephrectomized rat shows glomerular hypertrophy, glomerular hyperfunction, and the deposition of a high-molecular-weight substance in the mesangium in the early stage after nephrectomy, and the relationship between these features and the destruction of glomerular tissue in the advanced stage is now being discussed. Yoshida and Ichikawa have recently pointed out that, following subtotal nephrectomy, some growth factor may simultaneously induce glomerular hypertrophy and mesangial proliferation, the former leading to a disorder in the glomerular basement membrane or epithelial cells, resulting in a protein leakage, and the latter leading to glomerular sclerosis. Our present study shows that green tea tannin suppressed the leakage of urinary protein, suggesting that it delayed the progression of glomerular hypertrophy. In addition, the administration of green tea tannin reversed the proliferation of the mesangium in rats with chronic renal failure. It is probable that green tea tannin also inhibited one of the two processes described by Yoshida and Ichikawa, i.e., that leading to glomerular sclerosis resulted from mesangial proliferation that was induced by a growth factor working after nephrectomy.

On the other hand, it is considered that uremic toxins retained under renal dysfunction directly or secondarily affect renal tissue, leading to a deterioration of renal tissue and function, producing a vicious cycle that results in the terminal stage of kidney disease. In this connection, the present study shows that an increase in blood urea nitrogen and serum creatinine in nephrectomized rats was suppressed by green tea tannin. Considering this together with the previous finding that a renal failure-related accumulation of methylguanidine, which is the strongest among currently known uremic toxins, was significantly inhibited by green tea tannin, it is possible that green tea tannin improves the systemic milieu and eliminates the vicious cycle, thus inhibiting glomerular deterioration. In other words, the removal of a toxin that affects the renal function might have a beneficial effect on maintaining the cellular function.

The model for renal failure produced by partial resection of the renal parenchyma, as was used in the present study, shows hypertrophy or swelling of the remaining kidney tissue, with an increase in its weight. On the basis of the fact that the remaining kidney shows significantly increased oxygen consumption and enhanced ATP synthesis, Schrier et al. and Harris et al. have suggested that free radicals are involved in various ways in the occurrence and progression of renal failure. In the present study, the measurement of antioxidative enzymes involved in the elimination of free radicals such as superoxide and H₂O₂ revealed a significant decrease in SOD activity, and catalase and GSH-Px activity, suggesting that the free radical-scavenging system was destroyed in nephrectomized rats. To directly demonstrate this phenomenon, we investigated radical species by spin-trapping with 5,5-dimethyl-1-
pyrroline-N-oxide and found that the amount of the hydroxyl radical in the residual kidney tissue was greater than that in normal rat kidney. In contrast, the activities of SOD and catalase were significantly higher in those rats given green tea tannin after nephrectomy. Since these rats showed a weak action for GSH-Px, an enzyme which, like catalase, eliminates H₂O₂ and which is present in the mitochondrial matrix, the site of action of green tea tannin is speculated to be the peroxisome. Peroxisomes in rat liver contain flavin enzymes such as x-hydroxy acid oxidase and D-amino acid oxidase as well as Cu enzymes such as uric oxidase, and produce H₂O₂ during oxidation of the respective substrates. Through its action, catalase works to detoxify and utilize such H₂O₂ for cleaving long-chain fatty acids (a type of β-oxidation). The fact that tannin contained in green tea is able to increase the activity of this enzyme indicates that it has potential as a new therapeutic substance. However, since the radical species have not been determined, more detailed clarification of these issues is desirable.

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