The aim of this study was to compare the most commonly-used experimental models and to assess the microscopic renal changes in different models of cyclosporine A (CsA) nephrotoxicity. Wistar male rats were divided into five groups, eight animals in each. CsA was given in doses of 15 mg/kg, 25 mg/kg, and 100 mg/kg, respectively. The blood was collected for creatinine, urea, and uric acid levels analysis in the serum and the kidneys were sampled for microscopic examination on the 11th and 29th d of the experiment. CsA induced nephrotoxicity was characterised by increased serum levels of creatinine, urea, and uric acid. Microscopic features of CsA nephrotoxicity in all CsA experimental groups were observed. We would recommend the use of low doses of CsA for approximately 28 d as the most relevant experimental procedure for achieving the features of chronic CsA nephrotoxicity.

Key words: rats, cyclosporine A, nephrotoxicity, experimental model.
subcutaneously 25 mg/kg/d of CsA for 28 d (5, 9), group D – given subcutaneously 100 mg/kg/d of CsA for 10 d (6), and group E – given subcutaneously 1 ml/d of olive oil for 28 d.

CsA was dissolved in olive oil. The animals of groups B and D were a fed low-sodium diet. The animals in group D were euthanised on day 11 of the experiment, the animals in group A, B, C, and E were euthanised on day 29. The blood was collected for creatinine, urea, and uric acid levels analysis in the serum and the kidneys were sampled to evaluate the renal structure under a light microscope.

**Biochemical studies.** Creatinine, urea, and uric acid levels were measured using standardised kits for biochemical analysis (Cormay Diagnostic S.A., Poland).

**Morphological studies.** The kidneys were fixed in 10% buffered formalin and embedded in paraffin. After the removal of wax, 4 µm thick sections were processed and stained with haematoxylin and eosin (H+E), periodic-acid-Schiff (PAS), and Masson’s trichrome staining methods.

The following features, reported previously (13, 14), were assessed within the renal tissue: arteriolopathy, tubular injury, and interstitial fibrosis. Arteriolopathy of the afferent arterioles was manifested by the presence of eosinophilic, granular PAS positive circular or pearl-like deposits within their walls. Tubular injury was defined vacuolisation of the cytoplasm of tubular epithelial cells (TEC) with the presence of PAS positive deposits, degeneration, desquamation, necrosis, and sloughing of TEC, cast formation within their lumina, dilatation of tubules, thickening of tubular basement membrane (TBM), and tubular atrophy.

The assessment of renal injury was carried out in a blind fashion. A minimum of 10 fields for each kidney slide were examined for histological alterations.

**Statistical analysis.** Serum levels of creatinine, urea, and uric acid were analysed statistically by the parametrical Student’s t-test. Data were expressed as the mean ±S.E.M using STATISTICA 5.0 statistical software. The statistical significance was accepted for P<0.05.

### Results

**Biochemical studies.** The CsA administration impaired renal function in all treated animals. CsA induced nephrotoxicity was characterised by increased serum levels of creatinine, urea, and uric acid in comparison to the control group (Table 1), with creatinine level in group B being the highest of all CsA treated groups.

**Microscopic findings.** Microscopic features of CsA nephrotoxicity were observed in all examined groups. The kidneys of the animals of group C showed mainly tubular injury. It was manifested by vacuolisation of TEC cytoplasm with the presence of PAS positive granular deposits. Degeneration, necrosis with desquamation, and sloughing of TEC were seen within the proximal cortical tubules (Fig. 2). Tubular changes were patchy, involving multiple tubules in any given area. No arteriolopathy was seen in this group.

The microscopy of the kidneys of the animals in groups B and D showed tubular injury involving tubules in most of the examined areas in the animals of group D (treated with 100 mg of CsA/kg for 10 d) and being more patchy in group B. The microscopic features of tubular injury, besides vacuolisation and degenerative changes, were their dilatation, thickening of TBM, and atrophy (Figs 3, 4, 6). Arteriolopathy of afferent arterioles was observed in groups B and D. Hyaline thickening of arteriolar wall with the presence of PAS positive circular or pearl-like granules were seen in most afferent arterioles and focally within small cortical arterioles (Figs 3, 6). These led to the narrowing of afferent arterioles lumina.

As the consequence of these vascular changes, the collapse of capillaries within the glomeruli was seen and the Bowman’s space was dilated (Fig. 4). The interstitial fibrosis of renal cortex was seen in groups B and D but not in group C. It was manifested by delicate, stripped type fibrosis surrounding atrophic tubules (Fig. 5).

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (control)</td>
<td>0.879 ±0.022</td>
<td>52.63 ±3.0</td>
<td>1.703 ±0.09</td>
</tr>
<tr>
<td>B</td>
<td>1.64 ±0.29*</td>
<td>133.84 ±10.45*</td>
<td>3.84 ±0.69*</td>
</tr>
<tr>
<td>C</td>
<td>1.14 ±0.071*</td>
<td>109.09 ±9.805*</td>
<td>2.90 ±0.12*</td>
</tr>
<tr>
<td>D</td>
<td>1.3 ±0.089*</td>
<td>227.31 ±10.95*</td>
<td>2.81 ±0.20*</td>
</tr>
<tr>
<td>E</td>
<td>0.939 ±0.022</td>
<td>59.74±1.82</td>
<td>1.714±0.10</td>
</tr>
</tbody>
</table>

* P<0.05 vs. control group
Fig. 1. Group A. Normal histology of rat’s kidney H+E. 100x.

Fig. 2. Group C. Vacuolisation, degeneration, and necrosis of TEC within the renal cortex. H+E. 100x.

Fig. 3. Group B. PAS positive circular granules within the walls of afferent arterioles. Vacuolisation of proximal tubular epithelial cells. PAS. 200x.

Fig. 4. Group B. Vacuolisation and PAS positive deposits within TEC. Glomerular collapse with widening of Bowman’s space within the glomerulus. PAS. 200x.

Fig. 5. Group B. Delicate stripped-type fibrosis and atrophy of cortical tubules. Masson’s trichrome staining. 200x.

Fig. 6. Group D. PAS positive pearl-like granules within afferent arteriole. Dilatation of proximal tubular lumina. PAS 200x.
Discussion

The present study was carried out to assess the microscopic changes within the kidneys in the course of experimental CsA nephropathy in rats given different doses of CsA. The results obtained revealed that CsA administration results in the impaired renal function and the development of structural changes within the kidneys. Significant increases in serum levels of creatinine, urea, and uric acid was present in all CsA kidneys. Significant increases in serum levels of administration results in the impaired renal function and doses of CsA. The results obtained revealed that CsA experimental CsA nephropathy in rats given different.

Microscopic examination of the kidneys of animals treated with CsA and fed a low-salt diet revealed features described as chronic nephrotoxicity (3, 4). The most serious problems in the course of the chronic use of CsA are arteriolopathy of afferent arterioles of glomeruli and tubular atrophy/interstitial fibrosis that in consequence lead to chronic irreversible renal failure and may be the main cause of graft loss (5, 9). In our study, the kidneys of animals in groups B and D revealed circular or pearl-like PAS positive granules within the walls of afferent arterioles and focally of small arterioles within renal cortex. Some of glomeruli showed collapse of capillaries and widening of the Bowman’s space. Tubular atrophy and associated stripped-type fibrosis were present in kidney samples of both examined groups. These features of chronic CsA nephrotoxicity were more diffuse in the group receiving 100 g/kg of CsA for 10 d and had a tendency to be more patchy in the group treated with 25 g/kg of CsA for 28 d. The animals of group B showed the most significant biochemical changes and kidney morphology was consistent with CsA nephrotoxicity in this group. Due to the high morbidity in the group D (30% in the course of the experiment), we would rather not recommend this model as currently it is not reported to be in use. The dose of CsA given to animals for 28 d ranges from 10 mg/kg (8) to 25 mg/kg (2) or 30 mg/kg (5) but all the researchers are in agreement that the animals must be a given low-sodium diet. We also would recommend as the experimental protocol the use of low doses of CsA for approximately 28 d as the most relevant ones for achieving the functional and morphological features of chronic CsA nephrotoxicity.

References