Characteristic pathological changes in the glomeruli in diabetic nephropathy (DN) include expansion of the mesangial matrix and thickening of the glomerular basement membrane (GBM). Streptozotocin (STZ) induced DN in rats represents an excellent model for evaluation of drug treatment efficacy due to the progressive development of severe glomerular sclerosis and tubule-interstitial fibrosis. Additionally, this model reflects high similarity of the intrarenal enzyme distribution with the one in humans. Electron microscopy represents a unique method for analysing kidneys on ultrastructure level. The aim of this study was to confirm kidney changes in rats with STZ-induced DN and to estimate the effect of the perindopril (PER) treatment.

DM was induced by a single IP injection of STZ (60 mg/kg) in 75 Wistar rats. The control group received only IP injection of citrate buffer, pH 4.5. The rats with blood glucose levels \( \geq 11 \) mmol/L, under fasting condition were included in the study and were left in diabetic condition for 4 wks to develop DN. The diabetic rats (n=50) were randomly assigned to two experimental groups (STZ and STZ+PER). In the STZ+PER group (n=25), PER was administered (6 mg/kg/daily) from week 4 to week 12. Changes in kidney structures were analysed in a double-blinded manner. Glomeruli were carefully graded in a sequential manner, to avoid grading the same glomeruli twice. The detected changes by light microscopy were analysed and confirmed by TEM. Kidney tissue samples with a size of 1-2mm² after de-paraffinization and rehydration procedure were post-fixed in 1% (v/v) OsO4 for 1h and then embedded in Durcupan™ resin. Semi-thin sections were stained with Toluidine blue, while ultrathin sections obtained from ultra-microtome (PT-PC PowerTome, Ultramicrotomes, RMC Products), were contrasted in autostainer (QG-3100 Automated TEM Stainer, RMC Products) by uranyl acetate and lead citrate. Samples were then analyzed on TEM (Jeol, JEM 1400) attached to digital camera (Veleta TEM Camera, Olympus) and controlled by iTEM software v.5.2.

The renal tissue examination (STZ group, after 8 wks), have shown presence of a moderate degree of glomerulopathy characterized with basement membrane thickening, expansion of the mesangial matrix, arteriolar hyalinosis and insudative protein deposits that obstruct some of the capillaries (Fig. 1). The ultrastructure analysis have identified uneven fusion of the podocytes and widening of the mesangial matrix with GBM thickening due to deposition of basal membranaceous sclerotic material (Fig. 2, Fig. 3).

The detection of the observed changes on the ultrastructure kidney level outweighs the expense of EM for routine diagnostic purposes and adds its value as irreplaceable technique for diagnosis of renal diseases.
Fig. 1: Histopathological features of kidney from untreated diabetic rat at 12 wks with damaged glomeruli, thickened GBM, altered tubular epithelium with clear cytoplasm due to intracellular glycogen accumulation and areas of partial tubular dilatation. Sections were stained with PAS reagent. Magnification × 100 and × 200.

Fig. 2: Histopathological features of kidney diabetic rat treated with perindopril at 12 wks. Sections were stained with PAS reagent. Magnification × 100 and × 200.

Fig. 3: An electron micrograph from STZ-induced DN rats. Untreated diabetic rat at 12 wks., uneven fusion of the podocytes as well as widening of the mesangial matrix with sclerotic GBM thickening (magnification × 20,000).

Fig. 4: An electron micrograph from diabetic rat treated with perindopril at 12 wks., showing mild thickening of the GBM and regenerated podocyte (magnification × 15,000).