Neurodegenerative disorders are characterized by progressive loss of specific neurons in the central nervous system. Although they have different etiologies and clinical manifestations, most of them share similar histopathologic characteristics such as the presence of inclusion bodies in both neurons and glial cells, which represent intracellular aggregation of misfolded or aberrant proteins. In Parkinson’s disease, formation of inclusion bodies has been associated with the aggresome-related process and consequently with the centrosome. However, the significance of the centrosome in the neurodegenerative process remains obscure. In the present study, the morphological and functional changes in the centrosome induced by rotenone, a common insecticide used to produce experimental parkinsonism, were examined both in vitro and in vivo. Aggregation of γ-tubulin protein, which is a component of the centrosome matrix and recently identified in Lewy bodies of Parkinson’s disease, was observed in primary cultures of mesencephalic cells treated with rotenone. Rotenone-treated neurons and astrocytes showed enlarged and multiple centrosomes. These centrosomes also displayed multiple aggregates of α-synuclein protein. Neurons with disorganized centrosomes exhibited neurite retraction and microtubule destabilization, and astrocytes showed disturbances of mitotic spindles. The Golgi apparatus, which is closely related to the centrosome, was dispersed in both rotenone-treated neuronal cells and the substantia nigra of rotenone-treated rats. Our findings suggested that recruitment of abnormal proteins in the centrosome contributed to the formation of inclusion bodies, and that rotenone markedly affected the structure and function of the centrosome with consequent induction of cytoskeleton disturbances, disassembly of the Golgi apparatus and collapse of neuronal cells.