Systemic arterial hypertension (SAH) is a multifactorial clinical condition with significant public health implications. Excessive Na+ intake is linked to the development and progression of SAH, with potential harmful effects independent of hypertension. Cardiotonic steroids (CTS), such as ouabain (OUA), are classic ligands of Na+/K+-ATPase (NKA) and studies show that some SAH patients exhibit elevated plasma levels of endogenous CTS, particularly OUA. The role of endogenous OUA is not well understood, but it is speculated to affect long-term salt homeostasis and blood pressure. Elevated plasma Na+ levels may correlate with increased endogenous OUA and potential kidney damage. However, the combined effects of OUA and Na+ on kidneys are obscure. This study aims to evaluate how OUA interacts with a hypernatremic medium affecting LLC-PK1 renal tubular cells in vitro. LLC-PK1 cells were submitted to different concentrations of OUA, NaCl, and mannitol (osmolarity control) for 48 h and assessed for morphology and cell viability (MTT assay). Western blot analyses were conducted to examine NKA α1 and MAPK expression in these experimental groups. Cellular distress, observed via phase contrast microscopy, was pronounced in groups exposed to high OUA (100 nM), Na+ (>232 mM), and mannitol levels. The MTT assay confirmed reduced cell viability in these groups compared to controls, with significant enhanced viability in cells treated with 1 nM and 10 nM OUA, as well as those exposed to 80% or 100% Na+ medium + 1 nM OUA, but the same is not observed for mannitol. These results suggest that high concentrations of Na+ and OUA decrease cell viability, whereas lower OUA concentrations partially block the deleterious effect of Na+ overload. Preliminary protein expression analysis indicated a decline of NKA α1 and p-ERK1/2 (100 nM OUA). Ongoing investigations are examining proteins related to cellular stress, viability, and transport mechanisms.