INTRODUCTION AND AIMS: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disorder characterized by the presence of fluid-filled cysts destroying renal parenchyma, leading to end-stage renal disease. The drugs commonly used to counteract cysts growth cause many side effects. An emerging literature, based on in vitro and in vivo studies, focused its attention on the therapeutic properties of natural compounds, as polyphenols contained in olive leaves. Therefore, we aimed to investigate the effect of an extract of olive leaves (OLE), containing greater quantities of polyphenols respect to that of fruit, in proximal tubular renal cells carrying truncating mutation in PKD1 gene, with the goal of establish whether and how these polyphenols could mitigate cystogenesis progression.

METHODS: All experiments were performed using immortalized tubular renal cells obtained from human autosomal dominant polycystic kidney cysts, (ATCC®-WT9-12). In monolayer culture, untreated or treated with OLE, we evaluated cell viability by MTT assay and gene and protein expression by real-time-PCR assay and Western-blot analysis. DNA laddering approach was used to detect apoptosis. Functional studies were performed by transient transfection and chromatin immunoprecipitation assays. Intracellular calcium measurement was detected with spectrophotometer using a fluorescent probe. For cyst growth studies, cells were cultured in a collagen matrix and cyst size was elaborated with ImageJ software.

RESULTS: Our studies revealed that OLE decreased, in a time- and dose-dependent manner, WT9-12 cells viability. The calculated IC50 value was used for all experiments. Firstly, we observed a significant upregulation of p21 while CD1 levels were decreased. Functional studies revealed that OLE transactivates p21 gene promoter activity by an increased recruitment of Sp1 transcription factor and RNA PolIII on responsive elements of Sp1 sited on p21 promoter region. Interestingly, OLE reduced the expression of key mesenchymal markers, as N-Cadherin and α-SMA, countering fibrosis observed in ADPKD progression. Time course assays showed that OLE interfered with intracellular signaling mediated by high c-AMP levels in WT9-12 reducing PKA levels that, in turn, enhanced phosphorylated Akt, restored B-Raf inactivation, leading to down-regulation of phosphorylated ERK1/2, in agreement with reduced cell viability. Moreover, our results evidenced that OLE significantly mitigated the basal triggered apoptosis, as concomitantly demonstrated by the reduced DNA fragmentation, the increased expression of anti-apoptotic Bcl-2 protein and the decreased pro-apoptotic p53 and BAD protein levels. Furthermore, surprisingly, OLE treatment ameliorated intracellular calcium levels, suggesting that this compound, affecting many pathways involved in ADPKD pathogenesis, could interact with the primary cause of ADPKD. Finally, three-dimensional studies leading to cyst formation showed that OLE significantly inhibited growth and decreased size of cysts respect to untreated cells.

CONCLUSIONS: This study provided, for the first time, the evidence that OLE significantly interferes with most of the pathophysiological mechanisms involved in cyst expansion, suggesting its therapeutic use as coadjuvant to prevent or counteract ADPKD progression.