This study aimed to investigate the effects of juglone on the human bladder carcinoma cell lines TCC-SUB and RT-4 in monolayer and spheroid cultures. Cells were treated with juglone at 24, 48, and 72 h of incubation. The activity of caspase-3 was detected in vitro using a caspase-3 colorimetric assay kit according to the manufacturer’s instructions. The bromodeoxyuridine (BrdU) labeling index was used to determine the cells of the synthesis phase. The terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay was used to determine the death of cells in both the monolayer and spheroid cultures. The control group had a large S-phase fraction and many of the TCC-SUB and RT-4 cells nuclei were observed to be positive for BrdU. The dead cell count was higher in the TCC-SUB and RT-4 cell lines with juglone applied than in the controls. We conclude that juglone significantly inhibits the proliferation and induces the apoptosis of TCC-SUB and RT-4 cells in vitro. KEY WORDS: apoptosis, bladder cancer, cell line, juglone, in vitro