# Effects of 2,4-dichlorophenoxy acetic acid (2,4-D) on viability and apoptosis Of human dental pulp derived stem cells

**Abstract:**

**Introduction & Objevtive:** 2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic herbicide which is used around houses and gardens and also on golf courses, ball fields, parks, and in agriculture and forestry to control broadleaf weeds. The indiscriminate use of 2,4-D imperils human health and the environment. In the present study, the effect of 2,4-D on viability and apoptosis of human dental pulp derived stem cells are evaluated.

**Material & Methods:** Afetr collecting the teeth, pulp tissue was separated from pulp chamber, and digested in a solution 3 % collagenase type IV. Then, suspensions of the pulp tissues were transported into 25-$cm^2$ flasks and incubated in alpha modified minimum essential medium eagle (αMEM) supplemented with 15% fetal bovine serum at 37°C temperature, 95% humidity and 5% CO2. hDPSCs were seeded in αMEM and treated with 2,4-D from 0.1µM to 5mM for 48 hours. 2,4-D effect on cellular viability was investigated using MTT and trypan blue staining. Flow cytometry used to determine the distribution of cells in different stages of cell cycle based on cellular DNA Content. To investigate the possible mechanism(s) of cell death following 2,4-D treatment, DAPI sand AO/EB stainings was performed. Activity of Caspase-3/7, SOD, GPx as well as MDA level were assessed. All experiments were repeated at least 3 times from inipendent samples.

**Results:** MTT results showed growth rate incresae with 2,4-D from 0.1 to 100 µM, while concentrations higher than 100 µM and up to 10 mM of 2,4-D could reduce the viability of treated cell significantly. The population histogram showed that 2,4-D arrested hDPSCs in G1 stage of cell cycle. Elvaluating morphological changes via AO/EB and DAPI staining indicated the increase in early and late apoptotic cell number following 2,4-D treatment. Activity of Caspase3/7 was augmented in treated group while SOD, GPx, ALP enzyme

activities were diminished. Furturemore, the level of MDA was incresed considerably following 2,4-D exposure.

**Conclusion:** Our results demonstrated a biphasic effect of 2,4-D on hDPSCs; lower concentrations could intense cells growth rate while higher concentrations decrese viability of cells. 2,4-D was able to induce apoptosis on hDPSCs which is accompnied by oxidative stress and lipid peroxidation. This study provides a suitable in vitro model to evaluate the toxicity of chemical compounds on stem cells.

**Keyword:** 2,4- D, hDPSCs, Viability, Growth rate, Apopt