

**OP 21 - Apoptotic effect of *Clerodendrum infortunatum* L. root in 2D and 3D models of human Rhabdomyosarcoma (RD) cells**

R. A. U. I. Ranatunge<sup>1\*</sup>, P. Soysa<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo

**Introduction:** Traditionally, most of the *in vitro* testing of new anticancer drugs is carried out in two-dimensional (2D) culture platforms. Though this method is well accepted, recent research studies have suggested that three-dimensional (3D) culture systems represent more accurate *in vivo* cancer models compared to 2D cultures. Therefore, this research study was conducted to evaluate the induction of apoptosis by aqueous extract of *Clerodendrum infortunatum* L. root (AECIR) in 2D and 3D cultures of human Rhabdomyosarcoma (RD) cells to understand the biochemical responses and efficacy of the AECIR.

**Methods:** The generation of 3D cell spheroids was carried out using hydrogels-scaffolds-based method. The antiproliferative activity was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and the morphological features of apoptosis was observed with Ethidium Bromide/Acridine Orange (EB/AO) after 24 hours of AECIR exposure. The level of reduced glutathione (GSH) was estimated by Ellman's reaction.

**Results:** Analysis of the antiproliferative activity by AECIR exhibited an inhibition of cell growth by 50% at a concentration of 579.9, 298.4, 151.6 µg/mL in 2D culture and 738.3, 306.3, 105.3 µg/mL in 3D culture for 24, 48, and 72 hours, respectively. The images confirmed morphological changes such as cell blebbing, apoptotic bodies, and chromatin condensation in both 2D and 3D systems. The induction of apoptosis was also reflected by the decreased level of reduced GSH after 24 hours at a range of 70.4 – 41.7 µg/mL in 2D culture and 61.2 – 17.4 µg/mL in the 3D culture compared to negative controls, 71.6 and 78.7 µg/mL respectively.

**Conclusion:** These findings suggest that the 3D spheroids system was more sensitive to AECIR compared to the 2D system.

**Keywords:** 3D cell culture, *Clerodendrum infortunatum* L., 2D cell culture, Apoptosis, Human Rhabdomyosarcoma

\*Email: udeshikaranatunge@gmail.com