

## Silibinin mode of death in HepG2, and hela carcinoma cell lines

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**Background and Aims:** Silibinin is a major active constituent of silymarin, the mixture of flavonolignans extracted from blessed milk thistle. Recently, it has been reported that silibinin has anticancer effects in various malignancies. Apoptosis is a mode of cell death morphologically and biochemically distinct from classical necrosis. It characteristically affects scattered single cells that undergo cytoplasmic and nuclear condensation, coupled with cleavage of DNA into nucleosome-sized fragments. In this study we investigated the mode of death of silibinin in HepG2, and HeLa carcinoma cell lines.

**Methods:** The methods used for detecting mode of death were light microscopy (trypan blue staining) and fluorescence microscopy (propidium iodide(PI) and acridine orange(AO) double-staining). Treatment was carried out in a 25 ml culture flask with silibinin at IC50(half maximal inhibitory concentration) for 24 and 48 hour. The cells were then spin down. 10  $\mu$ l of fluorescent dyes containing AO (10  $\mu$ g/ml) and PI (10  $\mu$ g/ml) were added into the cellular pellet at equal volumes. Freshly stained cell suspension was dropped into a glass slide and covered by a cover slip. Slides were observed under UV-fluorescence microscope within 30 min before the fluorescent color starts to fade.

**results:** Striking apoptotic cellular changes were observed after 24 hour of treatment by light microscopy including cell shrinkage, masses of condensed chromatin adjacent to the nuclear envelope, membrane blebbing and cytoplasmic and nuclear fragmentation leading to the formation of apoptotic bodies. The results obtained with AO/PI double staining and differential scoring of treated HepG2, and HeLa cells (200 cells population) showed that there is a statistically significant ( $P < 0.05$ ) difference in apoptosis-positive cells, which indicates clearly that a time-dependent apoptogenic effect has occurred.

**Conclusions:** It could be concluded that silibinin was able to produce distinctive morphological features of cell death that corresponds to apoptosis.

**Keywords:** Silibinin; Mode of death; Double dye staining