

Letter to the Editor

DNA Fragmentation Is Not Associated with Apoptosis in  
Zerumbone-induced HepG2 Cells

Zerumbone is a cytotoxic compound isolated from the herbal plant, *Zingiber zerumbet* Smith, which exhibits antitumor activity [1-2], anti-inflammatory effects and possesses anti-proliferative potentials in a variety of cell lines [3-4].

DNA fragmentation indicates an early event of apoptosis leading to cell death due to the absence of new cellular proteins synthesizing for cell survival. Previous studies indicated that the cleavage of double-stranded DNA in apoptotic DNA degradation occurs via the activation of endogenous  $Ca^{2+}/Mg^{2+}$ -dependent endonuclease that specifically cleaves between nucleosomes to produce DNA fragments that are multiples of ~180 base pairs [5].

In order to investigate DNA fragmentation, we treated HepG2 cells with zerumbone (IC50:  $3.45 \pm 0.026$   $\mu\text{g}/\text{mL}$ ) in both dose-dependent (2, 4, 6 and 8  $\mu\text{g}/\text{mL}$ ) and time-dependent manner (4, 8, 12, 16, 24, 48 and 72 h). The assay was performed using the Suicide Track™ DNA Ladder Isolation Kit (Calbiochem, CA, USA), according to the manufacturer's instructions. DNA was analyzed using 1.5% agarose gel electrophoresis, observed under UV illumination and visualized using a gel documentation system (UVP Biospectrum HR410, USA). To further confirm the induction of apoptosis, the protein of zerumbone-induced HepG2 cells using Western-blotting indicated a low and high expression of Bcl2 and Bax proteins, respectively.

In conclusion, these results indicate that no DNA fragmentation in the human hepatocellular liver carcinoma (HepG2) cells was observed even in the presence of caspase-3 during apoptosis. Therefore, we hypothesize that not all compounds necessarily indicate fragmentation of condensed chromatin into several discrete mass in cell lines as *in vitro* condition.

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Behnam Kamalidehghan\*, Fatemeh Ahmadipour and Mohamed Ibrahim Noordin

Dept. of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

\*Corresponding Author; Tel.: (+603) 7967 4909; Fax: (+603) 7967 4964; E-mail: Behnam@um.edu.my