Letter to the Editor

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

erumbone 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 Ca²⁺/Mg²⁺-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: $3.45 \pm 0.026 \ \mu g/mL$) in both dose-dependent (2, 4, 6 and 8 $\mu g/mL$) and time-dependent manner (4, 8, 12, 16, 24, 48 and 72 h). The assay was performed using the Suicide TrackTM 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 furthur 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 necessairly indicate fragmentation of condensed chromatin into several discrete mass in cell lines as *in vitro* condition.

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REFERENCES

- 1. Kinghorn AD, Farnsworth NR, Soejarto DD, Cordell GA, Swanson SM, Pezzuto JM et al. Novel strategies for the discovery of plant-derived anticancer agents. *Pharm Biol.* 2003;41(Suppl):53-67.
- Singh CB, Nongalleima K, Brojendrosingh S, Ningombam S, Lokendrajit N, Singh LW. Biological and chemical properties of *Zingiber zerumbet* Smith: a review. *Phytochem Rev.* 2012 Mar; 11(1):113-25.
- 3. Murakami A, Takahashi D, Kinoshita T, Koshimizu K, Won Kim H, Yoshihiro A et al. Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: the α,β-unsaturated carbonyl group is a prerequisite. *Carcinogenesis*.2002; 23(5):795-802.
- 4. Weng HY, Hsu MJ, Wang CC, Chen BC, Hong CY, Chen MC et al. Zerumbone suppresses IKKalpha, Akt, and FOXO1 activation, resulting in apoptosis of GBM 8401 cells. *J Biomed Sci.*2012 Oct; 19(1): 86.
- Cohen GM, Sun XM, Snowden RT, Dinsdale D, Skilleter DN. Key morphological features of apoptosis may occur in the absence of internucleosomal DNA fragmentation. *Biochem J.1992 Sep;286(Pt 2):331-4*.

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