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LS-10-P-2028 NEW ANTIOXIDANTS IN THE PREVENTION OF UVB-INDUCED KERATINOCYTE APOPTOSIS

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Skin cells can respond to UVB-induced damage either by tolerating it, or restoring it through antioxidant activation and DNA repair mechanisms or, ultimately, by undergoing programmed cell death when damage is massive. Nutritional factors, in particular, food antioxidants, have attracted much interest because of their potential use in new preventive, protective, and therapeutic strategies for chronic degenerative diseases including skin inflammation (Vitale et al., 2013).
Hydroxytyrosol (HyT), a polyphenol present in virgin olive oil, well tolerated by organism after oral administration, shows a variety of pharmacological and clinical benefits such as anti-oxidant, anti-cancer, anti-inflammatory, and neuro-protective activities (Hu et al., 2014). These properties have been also reported for its synthetic derivatives. In fact, in an our previous study we have already demonstrated the HyT and its esthers anti-apoptotic effect against pro-oxidant chemical agents in different cell models (Burattini et al., 2013).
Here, the possible protective effects of HyT and its derivatives against UV-induced apoptosis were investigated in HaCat cell line. Human keratinocytes were pre-treated with antioxidants before UVB exposure and their effect evaluated by means of ultrastructural and molecular analyses. After UVB radiation, a known cell death triggers (Salucci et al., 2012), typical morphological apoptotic features, absent in control condition (Fig. A) and in HyT alone treated cells (Fig.B), appear. In particular, nuclear chromatin condensation (Fig. C, D) cytoplasm shrinkage and vacuolization (fig. D) can be observed at Electron Transmission Microscopy. Moreover, in control cells (Fig. F) and in the case of HyT administration alone (Fig. G) no labelled nuclei can be detected after TUNEL reaction, they diffusely appeared after UVB radiation exposure (Fig.H, I). An evident ultrastructural apoptotic patterns (Fig. E) and TUNEL positive nuclei (Fig. L) decrease can be observed when antioxidants were administrated before cell death induction. These data have been confirmed by molecular analyses (Fig. M, N, O). In fact, after UVB radiation, both intrinsic and extrinsic pathways appeared activated as well as PARP, a specific protein to identify nuclear damage directly correlated to apoptotic nuclear changes. Antioxidant compound administration before UVB-induced cell death showed an evident caspase activation decrease according to morphological analyses described above. In conclusion, our preliminary results demonstrate that natural antioxidant compounds are able to prevent apoptotic cell death in human keratinocytes exposed to UVB, attributing to these molecules an important role in preventing skin damage.

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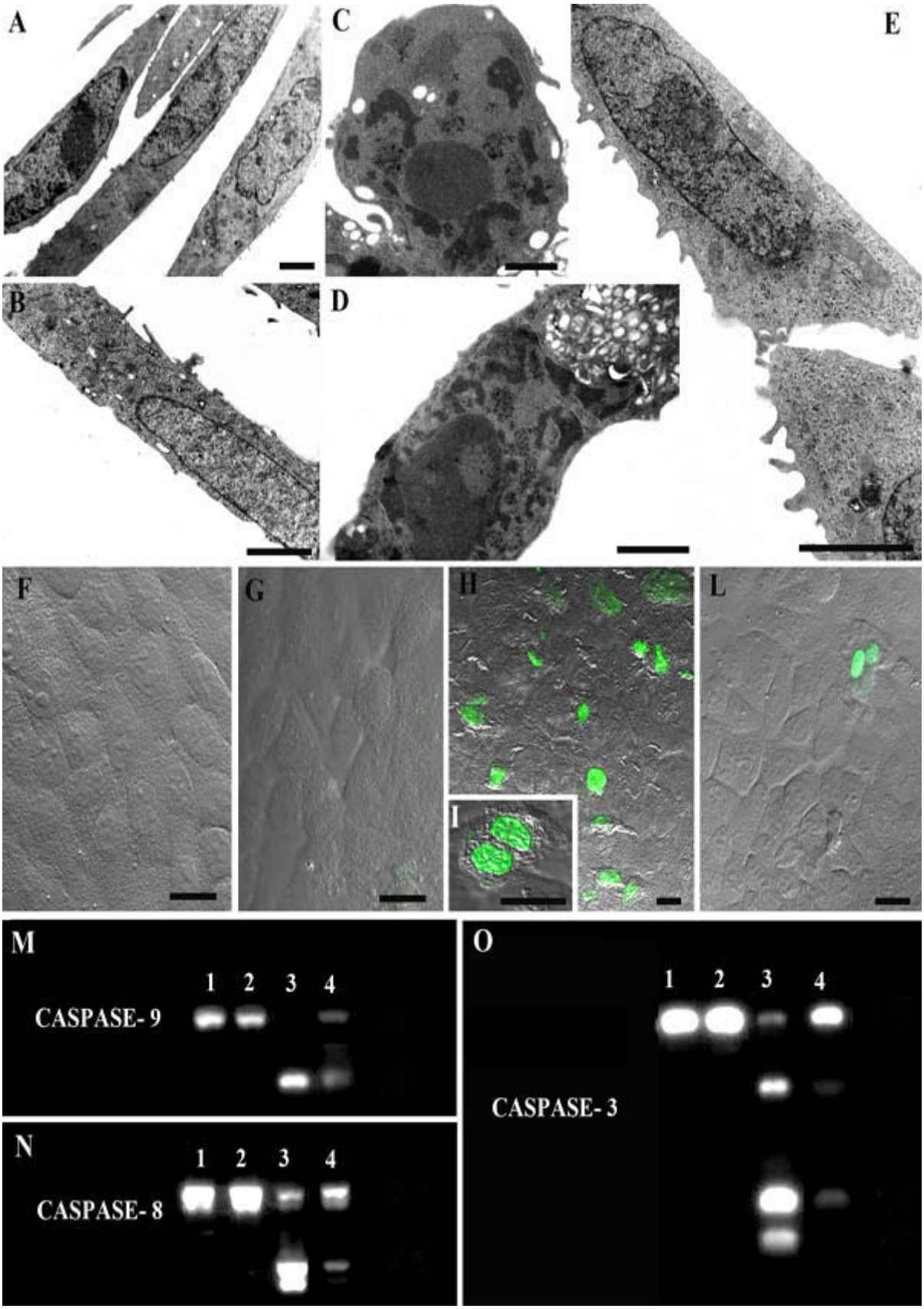

Fig. 1: Fig. 1: control (A,F,1) , HyT alone (B, G,2) , UVB (C, D, H, I,3), HyT+UVB (E, L,4) conditions, observed at TEM (A-E) and after TUNEL reaction (F-L). In M,N,O western blotting analyses of caspase- 9, -8 and -3, respectively. Bars: 2µm for A, B, E; 1µm for C, D; 25µm for F-L.