Cytotoxic and genotoxic effect of thymol derivatives on colorectal carcinoma spheroids

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Thymol is a monoterpene phenol with a characteristic odor. Thymol has potential uses in the pharmaceutical, cosmetic, food, agronomic industries, and so on. It has a proven bioactive effect on tumor cells, including colorectal cancer tumor cells.
However, its properties such as low solubility, absorption, and cell penetration prevent its wider application. Therefore, new hydrophilic derivatives – acetic acid thymol ester and thymol α-D-glucoside - were synthesized. In our study, we treated spheroids of colorectal cancer tumor cell lines (HT-29 and HCT-116) with thymol, acetic acid thymol ester, or thymol α-D-glucoside on a concentration scale for 24 hours. Cytotoxicity was determined by the MTT method. The genotoxic effect of substances was analyzed by the single-cell gel electrophoresis (comet assay).

For a comprehensive assessment of the effect of thymol and the newly synthesized derivatives - acetic acid thymol ester and thymol α-D-glucoside, the cytotoxic and genotoxic effect was also determined in 3D culture on colorectal cancer tumor cells. 3D cell culture ensures greater stability, while better representing real cell aggregation, morphology, and mutual cell interaction. As a result, the creation of a more complex microenvironment was ensured, which to a greater extent corresponds to the real conditions *in vivo*. Spheroids were formed after 5 days using ULA (ultra-low attachment) microplates. Subsequently, the cytotoxic and genotoxic effects of thymol, acetic acid thymol ester, and thymol α-D-glucoside were analyzed and compared using the methods mentioned above.

Our results demonstrated that a newly synthesized derivative - acetic acid thymol ester - with targeted chemical structure modification acts more effectively on both colorectal cancer cell cultures in 3D at much lower concentrations than thymol alone. Comet assays have shown a significant increase in DNA damage for the newly synthesized derivative even at non-cytotoxic concentrations. The HCT-116 cell line showed higher DNA damage values than HT-29. Incucyte Zoom noted the effect of thymol and acetylthymol on the proliferation of both tumor cell lines. The results confirmed our assumption that the newly synthesized hydrophilic derivative can act more effectively than thymol. In the future, we would like to focus on determining the expression of selected proteins using the Western blot method.

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