MUTAGENIC AND GENOTOXIC EFFECTS INDUCED BY CARVACROL, THYMOL AND THEIR MIXTURE IN CACO-2 CELLS

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Oregano essential oil has been selected by food industry as additive to develop new active packaging systems in order to improve shelf life of perishable products. The aim of this study was to evaluate the possible mutagenic and genotoxicity effects of carvacrol and thymol, main components of this essential oil, and their mixture. The mutagenic effects of carvacrol, thymol and their mixture were performed according to the recommendations of Maron and Ames (1983), and following the principles of OCDE guideline 471 (1997). Five *Salmonella typhimurium* histidine-auxotrophic strains were used for the assay. Different concentrations of carvacrol (29, 56, 115, 230 and 460 µM), thymol (15.6, 31.3, 62.5, 125 and 250 µM) and the mixture (29-2.9, 56-5.6, 115-11.5, 230-23, 460-46 µM) were assessed either with or without metabolic activation (S9) in three independent experiments. Moreover, the possible genotoxicity of carvacrol and thymol was evaluated in the intestinal Caco-2 cell line using the comet assay. Cells were exposed to carvacrol (115, 230 and 460 µM), thymol (62.5, 125, 250 µM) or their mixture 10:1 carvacrol/timol (75:7.5; 150:15; 300:30 µM) for 24 or 48 h. The standard comet assay and detection of oxidative DNA damage with enzyme (Formamidopyridine DNA glycosylase, FPG or Endonuclease III) comet assay were used. Regarding Ames test, thymol did not show any mutagenic effect at any concentrations tested, although carvacrol demonstrated a mutagenic effect in all concentrations tested. Similarly, the mixture of carvacrol-thymol showed mutagenic activity at 115-11.5 and 460-46 µM only in presence of S9 metabolic activation system. In the standard comet assay, the results revealed no significant increase in DNA strand breakage for both compounds and the mixture in any concentrations and exposure time. However, the Fpg-modified comet assay revealed a significant increase after 48h of treatment when Caco-2 was exposed to the highest concentration of carvacrol (460µM) and their mixture (300:30 µM). Therefore, considering that carvacrol and the mixture with thymol presented potential mutagenicity and oxidative damage in purine bases at the highest concentration assayed, further safety studies should be carried out before essential oils compounds could be widely used in foods.

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